UNIVERSITAT DE BARCELONA

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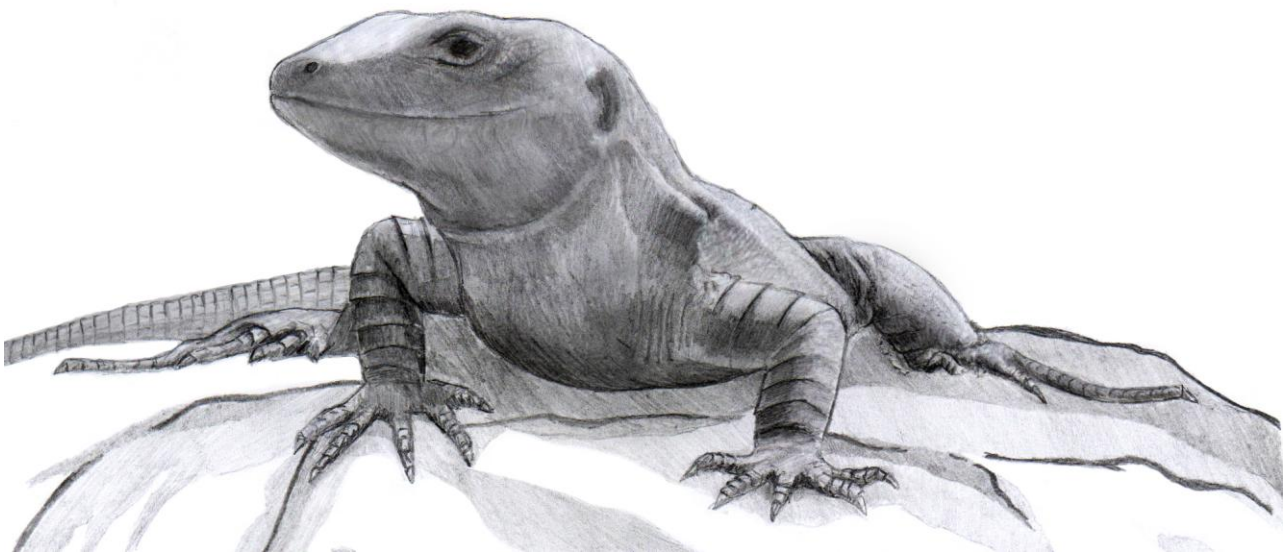
Thesis 2016



Universitat de Barcelona

IMEDEA CSIC

Programa de Doctorat en Biodiversitat (UB)

Institut Mediterrani d'Estudis Avançats

(Imedea, CSIC-UIB)

Evolutionary demography of the Balearic Wall lizard *(Podarcis lilfordi)*

La demografia evolutiva de la Sargantana Balear (*Podarcis lilfordi*)

Memòria presentada per Andreu Rotger Vallespir per optar al grau de doctor per la Universitat de Barcelona

Andreu Rotger Vallespir

Juliol 2016

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El director de la tesi
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Als meus pares i al meu germà

AGRAÏMENTS

Qui ho diria, però això s'acaba i és real, sembla que quan comences a fer la tesi tens molt de temps i ben lluny queda el moment que hauràs de començar a escriure la teva feina, ja ni pensaments el de plantejar-te com escriure els agraïments. Però com he dit, tot s'acaba i el temps passa molt ràpidament. Malgrat això, tot aquest treball no hauria estat possible sense el suport rebut d'una sèrie de persones i institucions que m'agradaria almenys anomenar. És impossible contar alguna cosa especial de tothom, però vaig intentar resumir-ho, ja que necessitaria un altre tesi per fer-ho!

En primer lloc vull agrair al meu director de tesis, Giacomo, muchísimas gracias por tu paciencia y por estar ahí siempre, hasta el último momento. Durante estos años he aprendido muchísmo gracias a ti y a tus consejos, hasta un poquito de italiano y de *Giacomés*. Sin ti no hubiera empezado todo esto. Y no puedo dar sólo las gracias a Giacomo sin Manolo, *Manolín*, no veas lo que he aprendido de ti durante esas horas de campo cogiendo lagartijas y lo que he disfrutado yendo a Pantaleu. Ha sido genial poder compartir el campo con vosotros dos, espero poder seguir disfrutando de esos islotes llenos de lagartijas que me enamoraron desde el primer día. los tres hemos hecho un buen equipo, o al menos lo hemos intentado. Y no podia faltar la *Botxor*, nuestro querido medio de transporte a los islotes, sin ella no hubieramos tenido la mitad de anécdotas que contar, y sin ella y sus averias no podría agradecer también al equipo de *ExcursionBoat* de la Colonia de Sant Jordi por estar siempre dispuestos a ayudarnos cuando necesitábamos una barca.

Gràcies també al finançament de la beca JAE-predoc (CSIC) que em va ser concedida i m'ha permès durant quatre anys dedicar-me a la investigació podent realitzar la meva tesi doctoral. Al Santi, el meu tutor, malgrat que estar lluny sempre ha tingut temps per ajudar-me quan li he demanat, i a Maria José, la responsable del doctorat, que sempre ha estat allà per respondre als meus dubtes.

D'altra banda, durant quasi 5 anys, moltes altres persones hi han contribuït a què jo ara estigui aquí escrivint aquests agraïments, algunes ja no hi són, moltes varen venir només de passada, d'altres es varen quedar més temps i unes poques han vingut per quedar-se. Però si som les experiències que vivim, totes aquestes persones han estat responsables en major o menor mesura de com sóc ara, de com seré en un futur i de què hagi pogut aconseguir tot això, i els estic molt agraït. M'haureu de perdonar si em deixo algú pel camí, però m'he de remontar l'any 2011 quan estava vivint a Madrid fent un Màster que vaig rebre un whatsapp de na Joana, *Juani, a tu t'ho he d'agrair el doble*, dient-me que la seva directora de tesis coneixia un investigador del IMEDEA que

cercava a qualcú per estudiar l'ecologia de les sargantanes, ni ho vaig dubtar, era la meva oportunitat, i així és com va començar tot.

I quin començament, no havia acabat el màster i ja vaig ser convidat pel Giacomo i els altres membres del grup d'ecologia de poblacions amb qui feria feina aquests anys i l'experiència va ser genial i ara segur que sóc poc objectiu però he de dir que és el millor grup d'investigació del món, des del primer moment et sents com en casa. Dani i Xell ha estat increïble poder conèixer-vos i compartir aquests anys amb vosaltres, a l'igual que poder estar al costat de n' Alejandro, Ana (mi super compi de despacho), Bibi, Noe (Hola xunga), Anita i Enric. I pels que han passat per aquest grup i ja no hi són, Mariana, Albert, Jose, Mike, Simone (mi otro super compi de despacho), Nikola, Lluïsa....i sense oblidar a Jon, muchas gracias por todo, parte de esta tesis es gracias a ti. Amb tots ells he compartit moltíssims moments a la tercera planta, reunions i *coffee breaks* al Agora del IMEDEA.

Moltíssimes gràcies a tots els altres IMEDEOS, sou un munt per nombrar-vos a tots, però en especial al grup d'ecologia terrestre, pels dinars i berenars i xerrades de qualsevol tema, gràcies Cris per tot, sempre tens un somriure per jo, Jaume que he de dir de tu que ja no saps ja som molts d'anys que ens coneixem, sembla que les nostres vides van en paral·lel. I gràcies Fran i David, heu arribat més tard però ja vós heu fet estimar molt. I d'altres persones que han estat per allà Rocio, Bea, Isa y Avi.

A la UIB, en especial al grup de genètica de la UIB, moltíssimes gràcies per acollir-me durant quasi un any i ensenyar-me. Gràcies a vosaltres he après moltíssim. Cori i Virginia gràcies per tenir tanta paciència i estar allà sempre que ho necessiti. Joana Maria gràcies per ensenyar-me a fer les extraccions, ets la millor profe del món, Joana (a tu et toca més abaix), Pepe, Barbara i Antònia que sempre s'han interessat pel que feia. Encara que no estigui dins el grup de genètica, però si que està molt vinculat, també vull agrair a Valentin, muchas gracias Valentin por tus consejos y sugerencias con todo lo relacionado con el campo y las lagartijas. Al grup de botànica, especialment a nen Biel que ens va ajudar a caracteritzar la vegetació dels illots. I gràcies a la fundació BIT, especialment a Óscar, Pep i Sergio, sense vosaltres, els "papás"del APHIS això no hagués estat possible, moltissimes gràcies.

A los "hueones" chilenos y gracias a la beca Jovenes Investigadores proporcionada por el banco Santander para poder hacer una estancia y viajar a Chile, gracias Mauricio por acogerme y hacerme sentir como en casa y a José que vívia en la universidad practicamente, gracias por esos cafés. Y a mis compis de piso, sobretodo a Achim y a Sonia, gracias por las excursiones y las salidas matutinas a correr por la ciudad.

To my Oxford partners, Tim and Sonya thank you so much for helping me during my short stay. Mark, Sam, Susan thanks for the Thursday evenings in the pub having pints, y a Emilie por ofrecerme asilo y enseñarme toda esta maravillosa ciudad.

Als amics "uiberos", el millor de la universitat, gràcies pels grans "momentassos" viscuts, els viatges, les rialles els "hello the new", i les biofestes i les verbenes. Barbara, Toni, Marina, Albert, Simó, Irene, Marilena, Joan, Raul, Jessy, Diana, Antònia i molta gent més, i especialment les meves dues nines preferides Joana, Juani o Flora i Maria Antònia, Fauna, és sort que em deixin formar part de les seves vides. I gràcies als personatges més personatges, Pep i Juame (surts repetit) sense vosaltres la meva vida seria molt més avorrida i ho sabeu, i Pau sense tu encara seria més avorrida, amb qui podria jugar a la consola?

A los amigos de toda la vida, a la trupe, que aunque nunca se han enterado mucho de que iba lo que hacía, ellos sabían que a mi me era lo que me gustaba y allí estaban, siempre me han apoyado y han estado ahí para cualquier cosa y desde hace muchos años, sin vosotros seguramente no hubiera llegada hasta aquí, os quiero mucho. Verito, Araceli (mis dos rubias preferidas), Maria, Lore, Victoria, Juli, Maria del Mar, Encarna, Laura y Lidia (las LaLis), Vicky, Javi, Encarna y sobre todo a mi primo Lluis, perquè duem junts tota la vida i el que encara ens queda per viure.

A tota la meva família, especialment als meus pares que sempre han estat allà, que sense entendre molt perquè feia això de "contar sargantanes" i perquè servia sempre m'han donat suport, sempre han estat allà des del primer moment, gràcies a ells dos i al meu germà Pau he aconseguit tot això. I sobretot he d'agrair poder escriure aquestes línies a Dani. Dani, el *artistasso* que ha fet la portada i els dibuixos, vares arribar tard a tot això, però gràcies per compartir amb jo aquesta darrera etapa, que de veres ha estat increïble, gràcies al teu suport incondicional això ha estat possible.

Contents

General introduction

Part 1

The emergence of evolutionary ecology

1.1. "Nothing makes sense...

"Nothing makes sense in Biology Except in the Light of Evolution" (Dobzhansky, 1973). This provocative phrase was first pronounced by the evolutionary biologist T. Dobzhansky and well resumes the attempt made during the 60s and 70s to integrate the classical Darwinian view of evolution with the growing field of molecular ecology. The synthesis of genetic and evolutionary theory, also known as the *synthetic view of evolution,* illustrates how different disciplines can complement each other providing a new theoretical framework to study the mechanisms underlying the diversity of life.

In recent years evolutionary biologists become increasingly interested on how individuals interact with the biotic and abiotic environment incorporating problematics and concepts typical of ecology (Fox, Roff & Fairbairn 2001). The merging of evolution and ecological concepts dates back to classic work such the one of the frequency melanism in Peppered moths (*Biston bitularia;* Kettlewell, Cadbury, & Lees, 1971) or studies of

heavy metal tolerance evolving in plants on mine tailings (Antonovics, Bradshaw & Turner 1971). However, the assumption that evolution happened too slow to be influenced by ecological changes gave an accepted wisdom that both ecological and evolutionary changes occur at different timescales (Slobodkin 1961). Over time, this perception changed and ecology was becoming increasingly important in evolutionary biology, with increasing evidence that evolution can occur fast, being able to occur at the same timescale of ecological ones, e.g. microevolutionary changes (short timescale evolution), leaving an ecological imprint (Carroll *et al*. 2007).

"*Nothing makes sense in Evolutionary Biology except in the light of Ecology" (Grant & Grant, 2008).* The above quote formalized in a sense the birth of *evolutionary ecology*, which assumes that ecological and evolutionary changes are linked and occur on the same timescale (Fox *et al*. 2001; Pelletier, Garant & Hendry 2009). Empirical evidence showing that ecological processes can lead to a rapid evolutionary change and vice versa was delayed mainly because experimental and analytical methods to measure selection were developed slowly. The arrival of recent advances in computational science, molecular and quantitative genetics; and methods that allow us to accommodate and integrate detailed long-term individual-based data and pedigrees have been critical to the consolidation of evolutionary ecology. Hairston, Ellner, Geber, Yoshida, & Fox (2005) were the first to propose a framework for measuring the contribution of evolutionary change and the contribution of ecological change, assessing the importance of both, evolutionary and ecological processes. Nowadays, the reciprocal interactions between natural selection (evolutionary dynamics) and environmental change (ecological dynamics) are well recognized into a conceptual framework known as eco-evolutionary dynamics (Fussmann, Loreau & Abrams 2007; Reznick, Ghalambor & Crooks 2008; Schoener 2011). This integration of ecology and evolution is a necessary step in our understanding of the

processes that shape and maintain biodiversity, the structure of communities and the function of the ecosystems (Post & Palkovacs 2009).

"*Nothing makes sense in Evolution or Ecology except in the light of the other" (Pelletier, Garant & Hendry, 2009).* Nowadays, there is little doubt that ecology and evolution interact reciprocally, something sometimes referred to as "eco-evolutionary feedbacks" (Turcotte, Reznick & Hare 2013). Now, the aim of this integrated approach is to understand the interactions between ecology and evolution and their relative contributions in population dynamics (Pelletier *et al.* 2009; Post & Palkovacs 2009; Ellner, Geber & Hairston 2011; Schoener 2011).

The dynamic interplay of ecological and evolutionary processes result from natural or anthropogenic forces (Lankau & Strauss 2007). Human actions provide a well example of selective forces that act fast, often promoting eco-evolutionary feedbacks in animal populations (reviewed by Reznick & Ghalambor 2001). Historical and recent human impact on the biosphere (global change, technology or human population growth) accelerates evolutionary changes in many organisms (Palumbi 2001; Grayson 2001). This impact is becoming a strong evolutionary force. Populations under direct or indirect influence of human activities are exposed to dramatic environmental perturbations "forcing" individuals to a rapid adaptive phenotypic change (Hendry, Farrugia & Kinnison 2008). Furthermore, human impacts commonly cause a change of the demographic environment for example by provoking a skewed age structure, a reduced life expectancy, or exacerbating fluctuations in population size (Oro 2014) having an eco-evolutionary link between individual phenotypic traits and demography (Ricklefs & Wikelski 2002; Coulson *et al.* 2006; Ezard, Côté & Pelletier 2009). The study of human global impact on the environment has been critical for the birth of evolutionary ecology, founded on the understanding of the eco-evolutionary feedbacks and how it works in natural populations. Thus, one of the main challenges of evolutionary ecology and of the recent

discipline; eco-evolutionary conservation, is to find out whether individuals can keep pace with the increasingly rapid changes in selection (Parmesan 2006; Hendry *et al*. 2008).

1.2. Eco-evolutionary feedbacks and levels of biological organization in evolutionary ecology

The interplay between ecology and evolution is evident at multiples levels of biological organization from genes to ecosystems (Whitham *et al*. 2006). Under the framework of eco-evolutionary dynamics, this integration at multiples levels seeks to link genetic and phenotypic variation, to population dynamics, biodiversity and ecosystem function in order to study contemporary evolution in community composition (Pelletier 2009). The effects of genetic or phenotypic variations have consequences on the population, community and ecosystem.

(Fig 1). These variations are made possible thanks to individuals, the target of natural (or human-related) selection (Mayr 1997). Individuals are heterogeneous and this difference can be expressed in a given trait value (phenotypic variability), which are in turn manifestations of underlying genetics and environmental variation. The consequence of this variation comes in the form of vital rates of survival, migration and reproduction (Coulson *et al*. 2006; Pelletier *et al*. 2007). In other words, individuals with advantageous traits can better survive and reproduce to pass their genes to the next generation, possibly changing the trait frequencies in the population. This influences the strength of selection on the trait considered, which may have direct or indirect effects on community structure and finally on ecosystem.

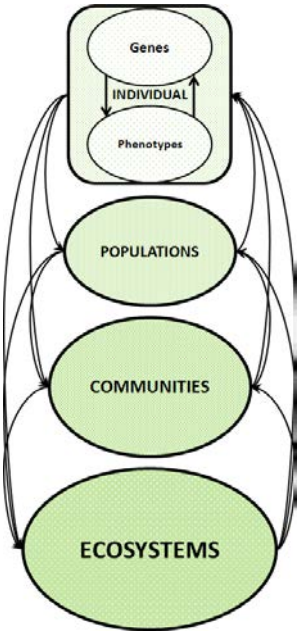


Fig. 1. Potentials feedbacks from genes to phenotypes across population, community and ecosystem levels of organization (modified from Bailey et al., 2009).

Palkovacs et al., (2009) showed that guppies *(Poedilia reticulata)* changed their life history traits according to predation pressure levels; this fact had direct influence in a different production of algal biomass that affected guppy individuals differently as well. Therefore, evolutionary changes caused by biotic factors (predation) have ecological effects (biomass production) and may feedback to influence the phenotypic traits of the focal organism. Ultimately, these feedbacks may intensify intraspecific divergences, which might end up in speciation (Hairston *et al.* 2005; Carroll *et al.* 2007).

1.3. The timescale of evolutionary ecology studies

Considerable evolutionary change can occur within and between populations in just a few generations (Hairston *et al.* 2005; Carroll *et al.*

2007). However, for eco-evolutionary dynamics to be meaningful, the impact of a (ecological or evolutionary) change must be large enough to cause a significant change in the other (ecology or evolution), and the strength of selection shows to vary greatly from year to year (Siepielski, DiBattista & Carlson 2009). If natural selection acts on a trait (i.e. body size) through survival or reproductive success, it will affect the size-dependent structure of the population and ultimately population dynamics, making selection to fluctuate within ecological time-scales and generating evolutionary changes, a process named contemporary evolution (Hendry & Kinnison 1999; Kinnison & Hendry 2001). However, in evolutionary ecology research, it is also important to take into account that evolution occurred in the past, since the processes occurred in the past have shaped the diversity that it is seen today. However, it is not possible to investigate these past processes directly, but we can explore possible past scenarios and a variety of evolutionary patterns both, abiotics (climatic fluctuations, increase or decrease of sea level) and biotics (historical predation). Under a phylogenetic context, the comparative method is used to derive insight into historical trajectories leading to contemporary patterns.

One the most popular examples of studies of evolution in the past using the comparative method is given by Losos (2009) and his study about ecomorphs of anoles lizards and their ecology and historical adaptive radiation on different islands, besides using phylogenetic approaches to explore patterns of coevolution of morphology, physiology and locomotor performance. Therefore, the integration between the reciprocal interactions of historical patterns (evolution) and current processes that can produce a pattern (ecology) is a necessary step towards major advances in our understanding of the processes that shape and maintain biodiversity.

Part 2

Evolutionary demography and the monitoring of animal wildlife populations

2.1. Evolutionary demography

The great phenotypic variability observed in animal populations is the result of the combining forces of genes and environment (Schlichting, Pigliucci & others 1998). Genotype-by-environment interactions are especially relevant to the study of phenotypic variation and selection in animal populations. One important environment that frames individual responses is the population itself, e.g. its age-structure (Coulson *et al.* 2001), its density (Tavecchia *et al.* 2005), the sex-ratio (Pérez-Mellado *et al.* 2015) and the level of competition (Tavecchia *et al.* 2007). It is therefore not surprising that eco-evolutionary feedbacks between phenotypic traits and demography are an important driver of micro-evolutionary processes (Ezard *et al.* 2009). Patterns of survival and reproduction determine fitness, and a wide theoretical framework links demography with evolution (Coulson *et al.* 2006; Pelletier *et al.* 2007; Rees & Ellner 2016). *But how exactly does evolution influence demography and vice versa?* The feedback arises due to a change in the state of the population, which alters selective pressures giving new phenotype distributions, which in turn, affects density that can affect all subsequent phenotype distribution (Coulson *et al.* 2006). This feedback might also be responsible of

cascading effects on multiple life-history traits of the species leading ultimately to a micro-evolutionary change (Reznick, Butler IV & Rodd 2001). The ability of individuals to alter the expression of a trait in response to environmental change, is assumed to determine correlations between environment and the phenotype observed at the population level. For example, Hairston et al (2005) using the long-term sequence of beak shape in Darwin's finch (*Geospiza fortis*), showed that varying ecological conditions in different decades impacted on the strength, direction, and outcome of selection and beak-size which contributed twice as much to variation in population size than ecological processes.

2.2. The uncomplete nature of field observations and the methodological advances

Hairston's study is an example of how long-term records of populations and individual-based information come into play and take significant interest (Clutton-Brock & Sheldon 2010). However, monitoring animal populations and individuals throughout their lifespan, to obtain long-term repeated measures of the expression of traits over a range of environmental conditions is not easy. In this respect, field biologists face many practical challenges. Probably the most important of these, is the non-exhaustive nature of the observations. Indeed in animal populations individuals might breed, move or die undetected. To draw conclusions from uncomplete observations it is necessary to apply methods for statistical inferences that specifically account for detection failures (Chapter I.1). The recent advance in statistical ecology has provided analytical frameworks in which detection failure is modelled as a parameter and used to obtained unbiased estimate of the demographic parameter of interest, such as survival, population size or movement probability (Williams, Nichols & Conroy 2002). The development of new methods has allowed a 'jump forward' in field techniques and in the refinement of the ecological questions to address (Chapter I.2 and

Chapter II). An example is the use of the non-invasive technique for individual recognition presented in Chapter I or the multistate analysis for the estimate of size-dependent survival presented in Chapter IV. In many cases, new answers provoke new questions that demand new methods, in a proliferous loop between eco-evolutionary biologists and statistical ecologists, in recent years, for example, computational advance allowed finer analysis of the genetic structure of the populations. A second example of this was the study of population history using the Approximate Bayesian Computation in Chapter III.

2.3 Merging genetic, field information and population dynamics

Studies on evolutionary demography aim to predict the future population directions and ultimately to investigate how contemporary population will respond to contemporary patterns of selection (phenotypic changes). An important aspect to address these questions has been the recent development in quantitative genetics (Rees & Ellner 2016). Quantitative genetics investigates the genetic relationships among individuals (parent–offspring, half-sibs, and so on) that are necessary to estimate the heritability of the ecological effects of organisms, which Hendry (2013) called 'ecological heritability'. Quantitative traits are measured in terms of continuous level. A change in the mean and the variance of a trait value of one generation (offspring) is a result of selection during the previous (parent) generation. This process can reiterate over generations to produce evolutionary change. This dynamics can be tracked using Integral Population models (IPMs; Tim Coulson, 2012; Easterling, Ellner, & Dixon, 2000) this recently developed methodology for evolutionary demographic studies (Rees 2016) integrate demography, genetic and the relationship between fitness-related components and phenotypic traits. An example is presented in Chapter IV.

Part 3

Evolutionary demography and the island syndrome

3.1. Islands as natural laboratory and human activities as an evolutionary force

Closed and small natural populations are suitable biological models for eco-evolutionary studies because the demographic closure and the limited spatial scale contribute to reduce the complexity of the ecological processes. In this respect, islands provide a good setting for eco-evolutionary studies and are often referred to as 'natural laboratories'. Indeed, the framing theory of evolutionary ecology and demography would not be what it is now without the studies on islands (Warren *et al*. 2015). Since MacArthur and Wilson (1963), islands have provided new insights to understanding fundamental processes in ecology and evolution and have played a key role in evolutionary theory (Darwin 1859; Lomolino 2005). Perhaps the most recent and significant insight has been to fill the gap between ecological and evolutionary studies, since isolated populations offer a good biological model to try to separate the relative contribution of these forces (Clegg *et al*. 2008; Clegg 2010). Moreover, in recently isolated islands such as the continental ones, the evolutionary processes triggered by the insular environment occur on contemporary timescales (Blondel *et al*. 1991; Thompson 1998; Schluter 2000; Stockwell, Hendry & Kinnison 2003; Whittaker & Fernández-Palacios 2007; Clegg

2010). Continental islands usually are geographically close comprising archipelagos where environmental conditions are similar and natural selection may be important when populations colonize these new habitats (Campbell & Echternacht, 2003; Velo-Antón, Zamudio, & Cordero-Rivera, 2012). Given the relatively simple ecological context, human perturbations on small islands act as an important selective force (Pregill 1986; Grayson 2001). However, there are little evidences and few rigorous studies about how historically human activities have affected wildlife populations. Pregrill (1986) showed that the historical human settlements in small close populations (islets) affected directly the phenotype of small vertebrates by selecting against large sizes.

3.2. The Island Syndrome: Characteristic growth, body size, and density.

Individuals on islands have a series of behavioural and morphological characteristics promoted by the absence of predators, the low resource availability and the reduced inter-species competition, which differ them from their mainland counterparts (MacArthur, 1967; Roughgarden, 1972; Adler and Levins, 1994). Theory on island adaptation predicts that small vertebrates on islands would tend to grow larger, have low fertility, survive longer and reach denser populations than their mainland relatives, a set of character shifts referred to as "Island Syndrome" (Adler & Levins 1994; Palkovacs 2003; Buckley & Jetz 2007). Although the Island Syndrome has been showed in many insular populations of mammals and birds (Adler & Levins 1994; Clegg & Owens 2002), it seems not to hold for lizards (Meiri 2007; Raia *et al*. 2010). Insular lizard populations reach high densities, however body size seems not to shift according to predictions (Meiri, Cooper & Purvis 2008). Lizards show a remarkable variation in body size across their populations. Such variability suggests that body size is a very plastic trait that responds to local selective pressures. In this respect tracking the dynamics of body size would help to identify local selective

pressures and individual adaptive responses. Part of this thesis has been devoted to investigate this variability in average body size in island populations (Blanckenhorn 2000; Badyaev 2002)

3.3. The case of *Podarcis*

The genus *Podarcis* (Wagler 1830) comprises the Wall lizards of Europe and Northwest Africa, with approximately 23 described species (Uetz et al. 2016) and nearly 300, mainly insular, subspecies have been considered (Böhme, Hutterer & Bings 1985). *Podarcis* is the most diverse reptile genus that inhabits the South of Europe (Harris & Arnold 1999). They can be found from Central Europe to the Sahara and from the Iberian Peninsula to the Crimea (Fig 2). It also one of the most important elements in Mediterranean ecosystems, playing a relevant ecological role in food webs (Carretero 2004). This group evolved and diversified in the Mediterranean Basin (Arnold, Burton & Ovenden 1978). The Southern European peninsulas and most especially associated archipelagos house the highest degree of endemicity and species richness. All *Podarcis* species are enormously variable and often remarkably plastic from a phenotypic point of view, both in coloration and in scale morphology and number.

Podarcis species look very similar to lizards of genus *Lacerta*, in fact they were considered a single genus until 1970s. However, it was shown that *Podarcis* form a distinct group differing from *Lacerta* by the construction of skull and the hemipennis, and by the development of caudal vertebrae. The systematics of *Podarcis* is still controversial, largely because the species are morphologically very similar, although exhibit substantial intraspecific variability (Arnold & Burton 1978). Molecular studies have placed the origin of the genus *Podarcis* during the Late-Middle Miocene in the early Tortonian (9-10 Mya) (Arnold, Arribas & Carranza 2007). Phylogenetic studies on *Podarcis* based on mitochondrial DNA (Harris et

al 1998, Harris and Arnold 1999) coincide in recovering the genus *Podarcis* as a monophyletic clade supporting the morphological observations of Arnold (1978). However, the intragenic relationship and the study of the geographical origin are fields complicated to cover because of the rapid colonization of the Mediterranean Basin and early diversification (Harris & Arnold 1999). Nevertheless, four geographical groups seem to be well supported: the Balkan group, the Italian group the Tyrrhenian-Balearic, and the Iberomagrebian group (see Carretero, 2008).

Fig. 2. Distribution of the genus *Podarcis* (modified from Arnold 1973).

The Balearic Wall lizard (*Podarcis lilfordi*, Günther 1874) belongs to Tyrrhenian-Balearic group, along with *Podarcis pityusensis, Podarcis filfolensis and Podarcis tiliguerta*. And with *Podarcis pityusensis, both* are medium-size lizards endemic of the Balearic archipelago, Spain. The origin of both species was during the re-flooding of the Mediterranean at the end of the Messinian Salinity Crisis 5.33 Millennia ago when sea level decreased and produced a direct connection between the Balearic archipelago and Iberian Peninsula (Krijgsman *et al*. 1999; Duggen *et al*. 2003; Brown *et al*. 2008).

Podarcis lilfordi, unlike *Podarcis pityusensis*, are not found on the main islands, as human introduced predators on Mallorca and Menorca relegated the Balearic lizard to surrounding islets and Cabrera archipelago, prompting its listing as 'Endangered' by the IUCN (Pérez-Mellado, V. & Martínez-Solano, I 2009).

Twenty-five subspecies are currently described and accepted inhabiting 42 different localities, later Terrassa et al. (2009) split *Podarcis lilfordi* in 4 different genetic clades that were fitted geographically.

Although the evolutionary process of the Balearic Wall lizard has been very complex, *Podarcis lilfordi* is a good model species to address eco-evolutionary questions. First, we know that due to historical events, this species originated and diversified, the phenomenon of vicariance (isolation by increase of sea level) is well documented, even the dispersal patterns to close islands. Furthermore, there is evidence of long periods of human interactions in some islands that might have generated specific selective pressures. In addition, many actual Balearic wall lizard populations, beside sharing a similar genetic structure and inhabiting comparable habitats, differ in density, coloration, body shape, body size and life-history traits (Salvador 1980, 1986). This variability is the necessary base to address question on its maintenance and origin (Fig. 3).

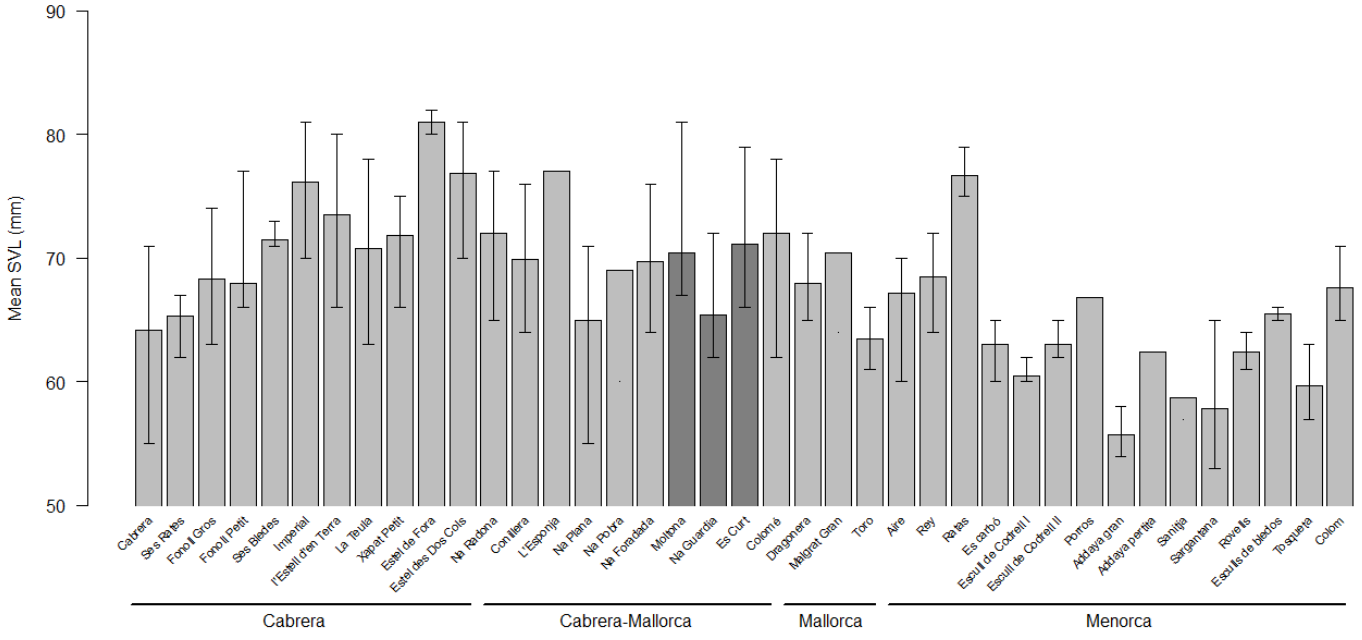


Fig. 3 Mean body size of males of Podarcis lilfordi from different insular populations. At the bottom populations are coupled in the genetic clades that (2009) found. The error bars show the range of sizes of each population, in dark gray the populations of this study. Bars without ranges no data available. (Data of body sizes: Salvador 1980, 1986; Böhme, Hutterer & Bings 1986).

Here, I have focused my investigations on three neighbouring isolated populations of this species, the Balearic Wall lizards, also known as Lilord's lizard. The three populations chosen are from the southern coast of Mallorca Island (Balearic Islands, Spain) belong to the same genetic clade and derived from the same recent colonization event: Na Moltona (hereafter, MO), Na Guardia (NG) and Es Curt (ES) (Fig. 4). I used 'soft' comparative methods to assess the relative contribution of ecological, environmental, and evolutionary processes that govern the dynamic of these three populations.

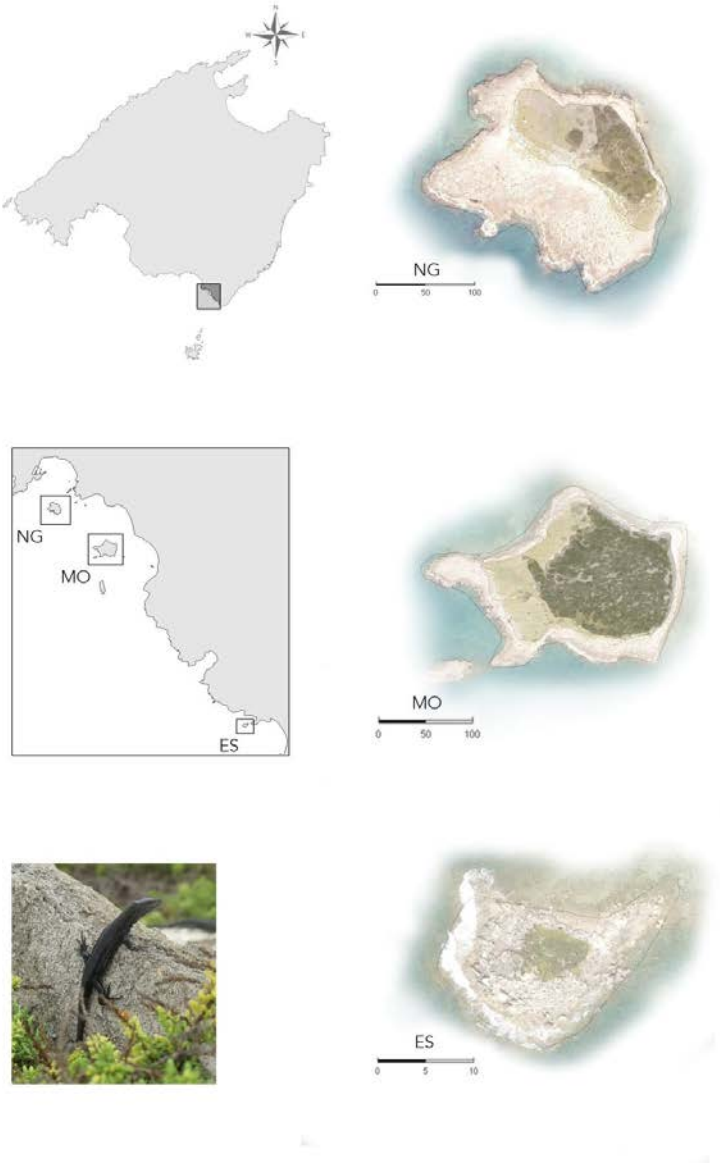


Fig. 4. Location of the study's islands and a individual from ES.

Objectives

Many topics in ecology and evolution are currently being studied under the framework of eco-evolutionary dynamics that is to say integrating genetic, ecological and demographic data. Here I was interested in investigating life-history adaptations of an endemic species inhabiting small islets where immigration and emigration are constraint. **The main objective of this thesis has been to shed light on the selective pressures imposed by the insular environment and the consequences of individual responses on the evolution of life-history traits.** Throughout this thesis I used techniques and concepts of ecology, allometric and genetic to develop an integrated population model that provides a new analytical framework to address eco-evolutionary questions and that unifies the ecological and genetic approaches in a demographic context (Fig.5). For five consecutive years (2011-2015), I participated in the collection of individual-based and environmental data. I first solved problems linked with the monitoring methods and in particular the imperfect sampling of natural populations (Chapter I), I then moved to the study of ecological predictors of body growth using individual-based data (Chapter II), I further investigated the inter- and intra-population genetic structure to describe the genetic architecture of each populations and their relationship (Chapter II). Finally, I merged all these results into an integral population model in which all demographic parameters were describes as a function of lizards' body size to develop an analytical framework to address eco-evolutionary questions (Chapter IV). The content of each of the four chapters in outlined below.

Chapter I: Monitoring Wildlife Population. Collecting individual-based data in natural population poses the problem of imperfect

detection. Indeed, animals might die, breed or move undetected. To solve this problem it is necessary to use statistical models to infer the biological processes or to obtain unbiased estimate of the demographic parameters from the raw observations. This chapter is divided in two parts corresponding two different publications presented in this Thesis. The first one is devoted to the development of innovative and not invasive techniques for individual recognition. In particular, I participated to the design and the test of new software, APHIS (Automated Photo-Identification Suite) for the semi-automatic identification of lizards. APHIS was specially designed to deal with sample sets of over a hundred photographs per field campaign (batches) and large image libraries. APHIS proposes two approaches for photo-matching that I helped to describe using real data from two capture-photo recapture studies, one on the Balearic Lizard, *Podarcis lilfordi* , and the other one on the Northern spectacled salamander, *Salamandrina perspicillata*.

The aim of the second part of this first Chapter is devoted to identify the strengths and weaknesses of the methods available to obtain an unbiased estimate of lizard density or population size through a 'soft' validation of the results.

Chapter II: Density-dependent and independent individual growth rate. Lizards are considered animals of continuous growth and not surprisingly the growth rate is an important life-history parameter with inter-specific and inter-population differences. A problem linked with the estimate of growth parameters in wildlife populations is that individual age is often unknown. Here, I used individual-based data to assess the length-at-age curve from capture–recapture data of uniquely identified and sexed individuals. For this I used the data collected in MO where the sample size was larger. Once the length-at-age relationship was described, I describe the influence of predictors of body growth. Mediterranean environment is classically considered as constant, however

variation in precipitation regime can profoundly affect summer drought and the biotic environment (Fig. 5). As possible predictors of body growth I considered population density and climatic conditions (temperature and precipitation) and quantified the relative role of population size and climatic factors affecting the body growth rate of juvenile, sub-adults, and adult lizards. I found that lizard density and temperature limited individual growth rate in juveniles but not in older individuals. In contrast, rainfall was an important constraining factor for the growth of both, juveniles and sub-adult lizards.

Fig. 5. Differences in the spring season in MO between two no consecutive years (2014 and 2016). Picture on the top was taken in April 2014. At the bottom picture of the same place in April 2016.

Chapter III: Inter- and intra-population genetic structure. In this chapter, I analysed patterns of divergence among the three populations using genetic markers, i.e. microsatellites, using 80 samples

from the tail tissues of lizards taken from the three islets. Once I established the genetic differences of the three populations I compared lizards' life-history traits such as body size, individual growth rate, fecundity, and survival probability. In one islet the average body size was about 14% lower than in the other two. I compared possible causes for differences in this important trait across populations. Once ruled out the possible role of genetic structure, biotic factors and demographic environment, the only plausible explanation for differences in body sizes were the historical events occurred in the past that may be relevant for the today population dynamics. The genetic results confirmed a recent bottleneck consistent with the historical presence of a Punic settlement about 400-500BC.

Chapter IV: Integral projection model. Integral projection models (IPMs) are a powerful analytical framework to tackle the influence of a continuous trait on individual fitness and population dynamics. Here, I developed an IPM model to compare the role of body size across the three different populations and its influence of each part in the life-cycle once established the size-dependent relationship of each demographic rate. Average fertility was not possible to measure in the field. Hence, in 2014 and 2015, I experimentally monitored the fecundity of 42 females brought at the University of Balearic Islands and subsequently released into their respective populations (approved by the Ethical Committee of the Balearic Government, license n.: 35/12/2015). To my knowledge it was the first time that a IPM is developed for a lizard species. I used the IPM results to make predictions of the demographic consequences of body size and age variations.

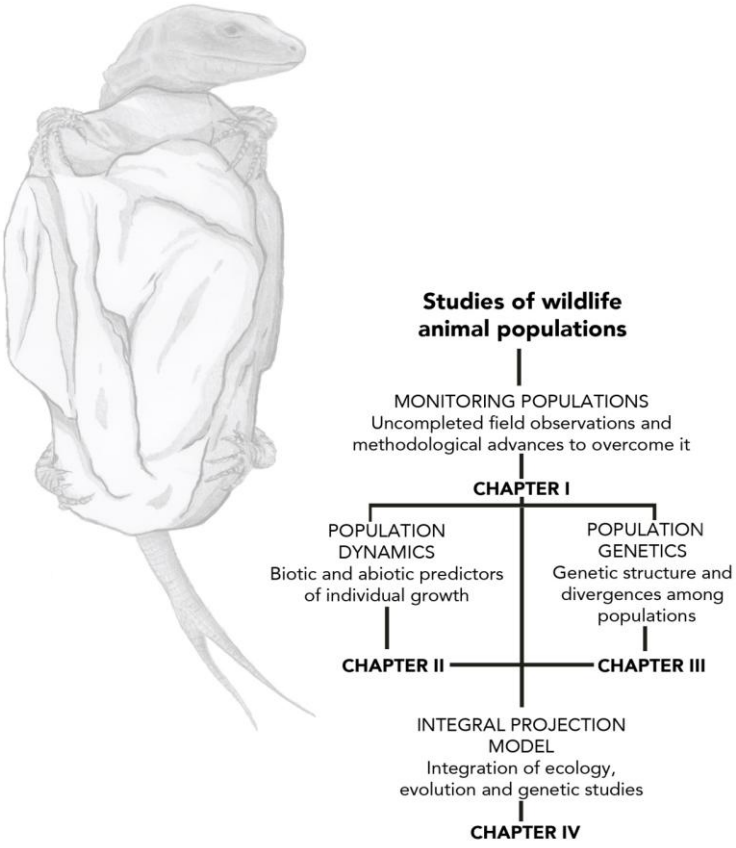


Fig. 6. Diagram showing the process followed by the study of the insular lizards

Supervisor's report

Report of the director of the Ph.D. Thesis in reference to its derived publications and the student's contribution to them.

Dr. Giacomo Tavecchia, Professor of Research at CSIC, tenured researcher of the Biodiversity and Conservation Department at IMEDEA (CSIC-UIB), Esporles, Mallorca (Spain), as PhD supervisor of the Ph.D. Thesis authored by Mr. Andreu Rotger Vallespir entitled: *Evolutionary Demography of the Balearic Wall lizard (Podarcis lilfordi).*

INFORM,

That the results and conclusions achieved in the research developed by Mr. Andreu Rotger as part of his Ph.D. Thesis have been organized in 4 chapters which correspond to 3 publications and 2 manuscripts (both in review round). Following, the list of publications and manuscripts is shown, indicating the journal impact factor IF (according to SCI of ISI Web of Knowledge, Journal citation Report-2015).

1. Moya, Ó.; Mansilla, P.-L.; Madrazo, S.; Igual, J.M.; **Rotger, A.**; Tavecchia, G. 2015. APHIS: new software for photo-matching in ecological studies. Ecological Informatics 27: 64-70.
   Impact factor: 1.683
2. Ruiz de Infante Antón, J.; **Rotger, A.**; Igual, J.M.; Tavecchia, G. 2014. Estimating lizard population density: an empirical comparison between line-transect and capture–recapture methods. Wildlife Research 40: 552-560.
   Impact factor: 1.49
3. **Rotger, A.**; Igual, J. M.; Smith, J. J.; Tavecchia, G. 2016. The relative role of population density and climatic factors in shaping the body growth rate of the balearic wall lizard (*Podarcis lilfordi*).

Canadian Journal of Zoology. 94: 207-215
Impact factor: 1.303

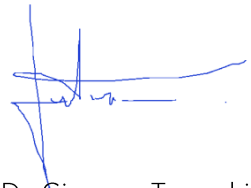
4. **Rotger, A.**; Igual, JM.; Genovart, M.; Ramon, C.; Rodriguez V.; Perez-Mellado, V.; Bibiloni, G.; Rita, J.; Tavecchia, G. Contrasting life-histories in neighbouring populations of lizards: the role of ancient human settlement. Review process in the Diversity and Distributions journal.
Impact factor: 4.566.
5. **Rotger, A.**; Igual, JM.; Tavecchia, G. Contrasting size-dependent life history strategies in female lizards from insular close populations. Review process in the Journal of Animal Ecology.
Impact factor: 4.827.

CERTIFY,

That Mr. Andreu Rotger contribution has been very active, as it is demonstrated by his participation in the definition of the objectives and the focus of the research and its derived manuscripts. Mr Rotger is first author in three of the final manuscripts and he has taken a major part and gave an important contribution on the others published papers.

Finally, I certify that any of the co-authors of the manuscripts detailed above has used, neither is going to use, implicitly or explicitly, the information produced and presented here with the purpose of elaborating another Ph.D. Thesis.

Esporles, 5 July 2016



Dr. Giacomo Tavecchia

Chapter I

Monitoring Wildlife Populations

Chapter I.1

APHIS: A new software for photo-matching in ecological studies

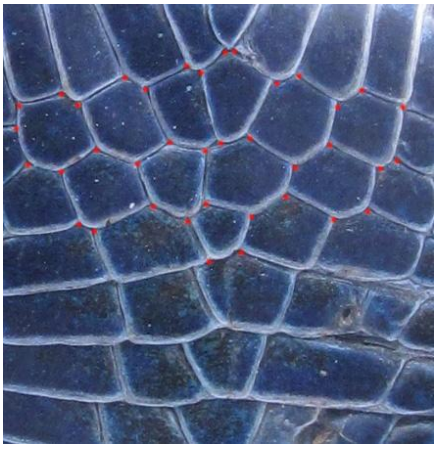
Chapter I.2

Estimating lizard population density: An empirical comparison between line-transect and capture-recapture methods

Chapter I.1

APHIS: A new software for photo-matching in ecological studies

Óscar Moya, Pep Luis Mansilla, Sergio Madrazo, José Manuel Igual, **Andreu Rotger**, Antonio Romano, Giacomo Tavecchia (2015). Ecological Informatics 27, 64-70.



APHIS: A new software for photo-matching in ecological studies

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Abstract

Unique body characteristics are increasingly used for individual recognition to avoid the effort and the potential negative effects of capture–mark–recapture technique. As a consequence there is a growing demand for computer procedures to assist users in photo-recognition of an individual. We present a new software for photo-matching developed to minimize the pre-processing time and maximize the speed of the matching procedure. In APHIS photos can be processed in batches of hundreds and users can select between two alternative matching procedures, one interactive, built as an extension of existing and freely available software, and one automatic. We assessed its performance in terms of individual recognition and time efficiency and illustrate its use with real capture-photo–recapture studies on a reptile and an amphibian species, the Balearic Lizard *Podiarcis lilfordi* and the Northern spectacled salamander *Salamandrina perspicillata*, with contrasting skin patterns.

Keywords: Photo-identification, Capture-recapture, Marking, Individual-based data.

Resum

Les característiques úniques de cada individu s'utilitzen cada vegada més per al reconeixement individual i així poder evitar l'esforç i els possibles efectes negatius de la tècnica de marcatge i recaptura. Com a conseqüència hi ha una demanda creixent de procediments analítics per ajudar als usuaris en el procés de foto-reconeixement dels individus. Aquí, es presenta un nou programa de foto-identificació que ha estat desenvolupat per reduir al mínim el temps de pre-processament i maximitzar la velocitat del procediment d'emparellament de fotografies. A l'APHIS les fotos poden ser processades en grups grans de centenars i els usuaris poden triar entre dos procediments alternatius d'unió: un interactiu i un automàtic. Es va avaluar l'efectivitat del programa en termes d'eficiència de reconeixement i temps individual per poder així exemplificar el seu ús amb els estudis reals de marcatge i recaptura en un rèptil i una espècie d'amfibi. La sargantana balear *Podiarcis lilfordi* i la salamandra del nord *Salamandrina perspicillata* respectivament, contrastant ambdues amb els patrons que tenen a les escames i la pell.

Paraules clau: Foto-identificació, captura i recaptura, marcatge, dades individuals.

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Introduction

Detailed data on individual life-history are used in ecological and evolutionary studies for the estimate of demographic parameters such as population size, survival and fertility of wildlife populations (e.g. Fernández-Chacón et al., 2011; Lebreton and North, 1993; Tavecchia et al., 2001, 2005; Williams et al., 2001). A common solution for the individual recognition of the animals is to apply a mark to the animal body in the form of a tag or a ring with a unique alphanumeric code. However, rings, tags, flipper bands or other marks can alter individual fates and behavior (Gauthier-Clerc et al., 2004; McCarthy and Parris, 2004). In addition to ethical issues (e.g. May, 2004), these negative effects lead to bias the estimates of the parameters of interest. As a consequence there is an increasing interest in using non-invasive methods for individual recognition, such as unique natural marks or body characteristics.

These methods have been applied with success in a wide range of taxa, in mammals (Karanth and Nichols, 1998; Langtimm et al., 2004; Martínez-Jauregui et al., 2012), amphibians (Gamble et al., 2008), reptiles (Sacchi et al., 2010), fishes (Speed et al.,2007; Van Tienhoven et al., 2007) or cephalopods (Huffard et al., 2008). However, with few exceptions (i.e. Perera et al., 2001), the photo-identification is restricted to those species featuring distinct colors, spots or marks. Photo-identification procedures consist of comparing a sample picture of an unknown individual with a library of candidate images of previously photographed individuals. This search is, in many cases, conducted by experienced observers who compare patterns and scars between photographs with the naked eye and might be extremely time-consuming when library contains hundreds of images (e.g. Martínez-Jauregui et al., 2012; Verborgh et al., 2009). Naked-eye comparisons are typically assisted by a preliminary grouping of the images using a multi-character score, for example by grouping images with a given chromatic pattern (e.g. absence or presence of specific marks, Carafa and Biondi, 2004). Unaided procedures may also become prone to errors when image libraries expand. There is now a growing demand in developing automatic or computer-aided procedures for photo-matching (Gamble et al., 2008). A computer-aided photo-identification system identifies the most probable sample–candidate matches, reducing the number of images to be inspected. Most photo-identification software solutions concatenate three processing steps. The first is a preprocessing step where a region of interest is selected and the image rotated, scaled or spatially corrected if required by comparison algorithms; the second is usually an automated comparison between the sample and the library of images,which arranges candidates by matching probability or likelihood values; a final step is a visual comparison of sample–candidate pairs for a limited number of plausible matches.

We present a new software solution, APHIS (Automated PHoto- Identification Suite), specially designed to deal with sample sets of over a hundred photographs per field campaign and image libraries containing more than a thousand samples. APHIS proposes two approaches for photo-matching, the Spot Pattern Matching (SPM) and the Image Template Matching (ITM). The

former has been built on the already existing I3S algorithm (Van Tienhoven et al., 2007) while the latter is a novel approach based on pixel matching that minimizes the user's preprocessing effort. ITM is a fast-running alternative to study species with apparent or easily recognizable spots or colored parts of the skin. The workflow and graphic interface of APHIS have been designed to reduce the time invested by the researcher in analytical tasks and to enhance user experience. We describe below the general features of the APHIS interface and illustrate the SPM and ITM procedures using real data from two capture-photo–recapture studies on the Balearic Lizard, *Podarcis lilfordi*, and on the Northern spectacled salamander, *Salamandrina perspicillata* (Fig. 1).

Material and methods

Automated PHoto-Identification Suite (APHIS)

APHIS (Automated PHoto-Identification Suite, freely available at http://www.imedea.uib-csic.es/bc/ecopob/) v. 1.0 combines C++ and Java modules. The idea behind APHIS was to provide users with a flexible environment for photo handling and matching. The Graphic User Interface (GUI) has been programmed using the Nokia Qt framework (http://qt.nokia.com/). The image preprocessing and analysis of the ITMapproach implements functions fromthe openCV v. 2.2 libraries (Bradski, 2000). The two available approaches, SPM and ITM, differ in how they treat andmatch the sample pictures. They performdifferently depending on photo and species characteristics (see below). APHIS also implements a metadata based filtering system for its SPM approach, a feature present in other photo-identification software solutions, i.e. Manta 2.1 and Contour 3.0 versions from the I3S series (http://www. reijns.com/i3s/). This function allows the user to predefine speciesspecific descriptive features and their possible alternative values forcharacterizing each sample. For example, a commonly useful feature would be the sex of the individual. A filtered search will only be conducted among sample–candidate pairs having equivalent character values and will substantially reduce the photo-matching time. Finally, an important feature in APHIS is the automatic creation of log files that register the score lists

obtained at each comparison. It also produces a registry of the matches validated by the user, which will lead to an easy analysis of capture–recapture data.

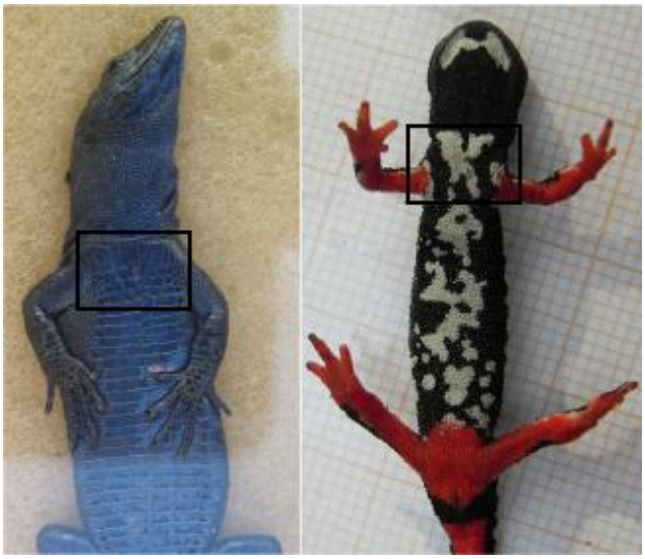


Fig. 1. The ventral side of a Balearic lizard (left) and of a Northern spectacled salamander (right). The black rectangles mark the region used for individual photo-recognition.

The Spot Pattern Matching approach (SPM)

The Spot Pattern Matching approach is based on the algorithm implemented in the freely available software I3S (Van Tienhoven et al., 2007). Each sample picture is pre-processed by the user before the photo-matching. During pre-processing the user delimits the region of interest with a given number of unequivocally identifiable reference points (typically three) and marks the set of spots within that will be compared during the matching of the defined area (Fig. 2; see Sacchi et al., 2010; Speed et al., 2007; Van Tienhoven et al., 2007 for practical examples). The coordinates of each spot in the space delimited by the reference points

form a fingerprint-like statistic. At the matching step, the spatially-corrected cloud of spots from the sample is compared with the fingerprints stored in a repository. This correction is the result of an affine transformation of the sample pattern mapped onto that of candidate one. Matching scores are calculated as the sum of metric distances between spots from every pair created in a sample–candidate comparison divided by the square of the total number of spot pairs. Lowest scores point to likely matches, being the number of spot pairs used during calculation relevant to the resulting score value (Fig. 2), although it is not yet clear as to what extent (Speed et al., 2007). Sacchi et al. (2010) used a range of 20 to 40 spots per image and found a negative association between the matching score and the number of points, but this effect was not large enough to impair matching results. The matching algorithm used by SPM approach in APHIS was directly extracted from the I3S Classic source code in accordance with its developers respecting its license agreement (GNU Public License v2). APHIS uses the exhaustive search version of the comparison algorithm described at Van Tienhoven et al. (2007). The exhaustive search uses every possible three spot pairs as reference points for different affine transformations, and not only those defined by the user (quick search). Score values are calculated for each transformation in comparison with the candidate and the lowest score is kept as final result. Exhaustive searches, although computer resource consuming, proved to be far more accurate than simple ('quick') searches (Van Tienhoven et al., 2007). Differently from the I3S software, the pre-processing and the matching phases in APHIS occur separately. This permits to process sequentially a group of samples and then launch the matching calculations for the whole set. Once the automated matching is finished, the user is presented with a list of sample–candidate alternatives ordered from lower to higher matching scores (Fig. 3). If multiple pictures from the same candidate are available, APHIS only shows these with the lowest score. Finally, the user should inspect the possible candidates and accept

the candidate as a recapture or discard the matching and register the sample as a new individual in the repository.

The Image Template Matching (ITM) approach

The Image Template Matching approach has been conceived to minimize the time invested by the user at the pre-processing step. It implements the match Template function of the Open Computer Vision libraries (OpenCV, Bradski, 2000), a pre-programmed function that slides a template image patch over an input image looking for matches. This method provides three different algorithms and their normalized versions in order to calculate a matrix of likelihoods of match per comparison. APHIS implements the normalized version of the correlation coefficient algorithm, which is the most accurate of the three (Bradski, 2000). Normalization is recommended to minimize the effect of lightning differences among template and input while calculating matching scores (Bradski, 2000).

During the ITM pre-processing step the user selects only two reference points for each picture (Fig. 4). It is extremely important to use small, spot-like and easily recognizable parts or species-characteristic natural marks as reference points. The reliability of matching scores will depend on the reproducibility of this selection across pictures. APHIS automatically transforms to gray scale, rotates and resizes the images aligning the reference points along a horizontal axis. Next, a pattern, which is the region containing the natural marks used for identification, is cropped from the sample images (Fig. 4). The result is a rectangular area delimited by the pixel distance between reference points as base and a height 105% of this distance.

The rectangle bottom is placed with a number of pixels below the reference points equal to 10% of the horizontal distance between them. A scale factor is applied to the resulting images such that all patterns finish

aligned by their reference points and with a fixed resolution of 460 × 436 pixels. Finally, six templates of 91 × 103 pixels are homogeneously cut out from the pattern, distributed in two non-overlapping rows and three non-overlapping columns (Fig. 4). Using six non-overlapping templates the effect of local image defects has less impact on the final score, enabling real matches to be well positioned in the score list ahead of random ones. It has to be noted that values used to delimit patterns and templates are not arbitrary; they were expressly set to delimit and subdivide the region of the animal that includes the natural marks. In the case of the common wall lizard, *Podarcis muralis*, for example, this region is the pectoral area which includes a scales pattern characteristic for each individual (Fig. 1; Sacchi et al., 2010). Note that the rectangular area is fixed in the current version of APHIS (460 × 436 pixels) to fulfil the needs of the current studies; however it can be set to any arbitrary value. At present this can be done only by changing the source code (line 12–16 of the source code 'ITM.cpp') but further development of APHIS will make it possible to set the area directly using the GUI. The six resulting templates extracted from a single sample are individually compared with the candidate pattern and the scores resulting from the comparisons are added up to produce the final sample–candidate matching score. APHIS produces an ITM score list per comparison where candidates are ordered, from highest to lowest, by their matching likelihood (the final score). Individual template scores range from −1 to 1, this being the score obtained when the template is a portion of the own input image. Therefore a value of 6 would be a perfect sample–candidate match.

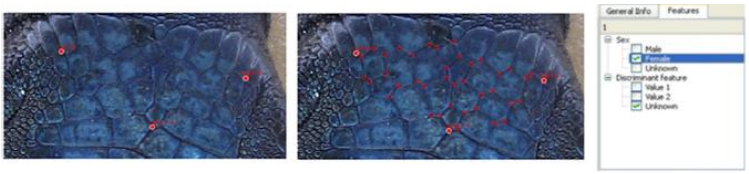
The capture-photo–recapture studies

We used real data from a capture-photo–recapture study on the Balearic lizard and on the Northern spectacled salamander to assess software matching performance in terms of individual recognition and time efficiency. Both studies aimed to estimate survival and population size using longitudinal data collected during multiple capture photo–

recapture sessions (e.g. Ruiz de Infante Antón et al., 2013; Tenan et al., 2013; Williams et al., 2001). The ventral region of both species is highly variable and preliminary studies have shown that the ventral patterns can be sued for individual recognition (Carafa and Biondi, 2004; Perera et al., 2001). In the Balearic lizard (dark morph) the ventral region is characterized by a uniform dark-blue or dark gray color (Fig. 1) and individuals differ in the position and dimension of their ventral scales. The ventral region of the spectacled salamander has white, black and red areas of variable shapes and dimensions (Fig. 1) with marked differences across individuals in the color patterns. Lizards were captured at the island of Moltona off the southern coast of Mallorca (Balearic archipelago, Spain) for three consecutive days in two sessions, June and October 2010 with pit fall traps positioned along and inside shrubs within an area of c. 0.21 ha (Ruiz de Infante Antón et al., 2013; Tenan et al., 2013). Captured individuals were held under a glass to ensure a clear picture of their ventral scales (Figs. 1, 2, 3 and 5). Photos were taken using a digital camera (Canon© G10) fixed to a stand and positioned inside a photo-cube to standardize light conditions. The picture was made after aligning lens marks with the collar of the individual to diminish differences in rotation, translation or lighting across the pictures. After manipulation, lizards were released. To assess the performance of the photo-identification method, all individuals were double-marked using a low-temperature medical cauterizing unit (Winne et al., 2006). Images of the Northern spectacled salamander have been collected in an area of c. 1 ha of the "Monte di Mezzo" Natural Reserve as a part of a large-scale ecological study (MANFOR CBD; LIFE09 ENV/IT/000078). Animals were captured by hand during their terrestrial activity in two sessions of two consecutive days twenty days apart (8–9 and 28–29 October 2013).

Images were taken at low resolution (1280 × 960 pixel) using a digital camera (Nikon© Coolpix P100), at variable distance from the subject and without standardizing light conditions. Individuals were first identified by

assigning a binary code to each image on the basis of four chromatic characters as suggested in Carafa and Biondi (2004). These results were used to assess the percentage of correctly identified matches by APHIS.



(1) (2) (3)

Fig. 2. Preprocessing for the SPM approach is divided into two steps: (1)manual selection of three reference points and (2)manual selection of between 30 and 50 intersections. Optionally, a third step (3) can be applied if the user decides to use individual discriminant characters to reduce processing times and to improve the quality of the resulting candidate list by constraining the analysis within individuals that show a given character.

Given the belly pattern of salamanders and the absence of clearly identifiable spot-like points, the ventral images were processed using ITM approach, only.

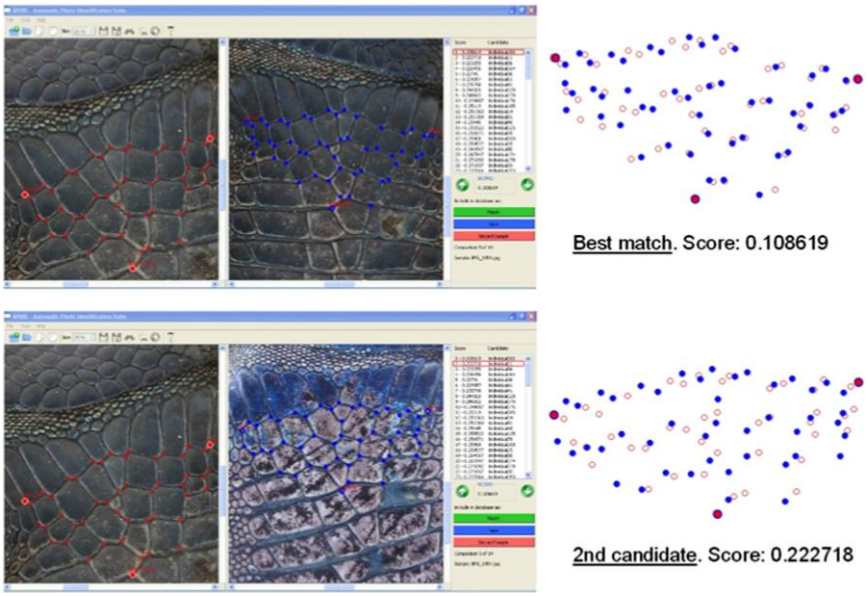
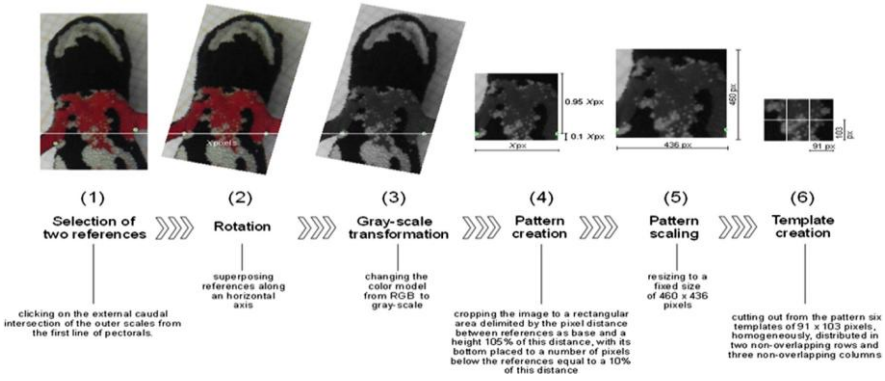


Fig. 3. The affine transformation of the fingerprint happens during the matching step and is comparison-dependent as far as it is applied for each sample–candidate pair under analysis. This figure shows the superposition of sample–candidate fingerprints for the first (best match) and second candidates of a SPM comparison from our study. It also includes two screen-shots of the APHIS display during the visual inspection of both comparisons. The sample is situated to the left of the screen, the candidate in the middle part and the controls used for decision-making to the right. The area reserved to display images has been maximized to show a general view of the animal that can be of help during the identification.

In each study the images taken the first day were used to create the initial repository. APHIS classified each subsequent photo as a recapture or as a new individual whether a match was found in the existing catalog or not, respectively. When a match is found the processed image is stored in the same directory of the matched sample, otherwise a new directory is created. Each processed image is considered as candidate for next comparisons, so that multiple images from the same individual taken in different capture-photo–recapture sessions are treated as independent samples. The reliability of both approaches implemented in APHIS was assessed by recording the number of correctly classified recaptures. For

each misclassified picture we assessed the phase in which it occurred and inspected photo characteristics to identify possible physical character responsible for the misclassification. In addition to the real sets of image, to evaluate time efficiency of the SPM and ITM approaches we randomly choose 100 samples of Balearic lizard and analyzed them against a repository of a thousand candidates in a computer with an Intel CoreDuo 2.40 GHz process or with 3 GB of RAM memory. In general, SPM approach required between 30 and 50 points per individual and, in both approaches the 20 first candidate pictures proposed by APHIS as potential matches were inspected before assignment. We have calculated the total time invested by the user in supervised tasks of this analysis, which include the pre-processing steps (i.e. the marking of scale intersections in SPM approach) and the visual comparison of likely matches after the processing phase occurred.

Fig. 4. Preprocessing for the ITMapproach is divided into six steps: (1)manual selection



of two references, (2) references are aligned with the horizontal axis, (3) the RGB image is transformed to gray-scale, (4) a region of interest is selected, (5) the image is resized to a fixed resolution, the resulting image is the pattern and (6) six non-overlapping contiguous templates are extracted automatically from the lower half of the pattern. Steps (2) to (6) are automatically performed by the software and do not need supervision.

Results

The 287 images, of which 91 were recaptures, were analyzed using the SPM and the ITM procedures. The two approaches, ITM and SPM, delivered similar results, however, the overall number of photos classified as new captures by both approaches were different. The SPM approach correctly classified all newly photographed individuals (percentage of correctly classified pictures = 100%), while ITM found 85 of the 91 recaptures (93.4%). Excluding user's mistakes (e.g. reference points placed wrongly), ITM approach resulted in 95.6% of correctly classified recaptures. The three errors were due to image characteristics such as marked differences in luminosity, local shadows, or variations in pigmentation or lepidosis, i.e. the scale pattern deformation due to body torsion. Nineteen of 305 images of northern spectacled salamander were recaptures. In this case the ITM approach correctly classified them all (100%). The whole ITM analysis took 52 min against the 215 min of the non-automatized method originally used to determine the number of recaptures. The analysis of 100 recaptures on a repository of a thousand took a total of 329 min with ITM and 266 min with SPM (Table 1), done in separate intensive batch sessions. However, the time invested in supervised tasks for the ITM approach (104 min) was nearly a half of this invested at the SPM approach (197 min; Table 1). The unsupervised task took 225 min for ITM and 69 min for SPM. Hence the ITM approach had a longer processing time but the pre-processing phase was faster. On the other hand the longer preprocessing phase in SPM was paid off by a short processing period.

Approach	Time (min.)		
	Total	Supervised tasks	Unsupervised task
SPM	266	197	69
ITM	329	104	225

Table 1. Time spent in photo-matching of lizard images using a batch search of 100 samples against a repository of 1000 individuals. Supervised tasks include pre-processing (reference points and pattern edition) and post-processing (visual inspection of likely matches); the calculation of scores for each sample–candidate pair is automatically conducted by the software, therefore it is an unsupervised task.

Discussion

Individual identification by photo recognition is becoming an increasing area of research. At present, there are several routines available for photo-matching, for example 'I3S' (van Tienhoven et al., 2007),'MantaMatcher' (Town et al., 2013), 'StripeSpotter' (Lahiri et al., 2011), Sloop (Gamble et al., 2008) and 'Wild-ID' (Bolger et al., 2012). Some are highly customized and some are very flexible. Our purpose here was neither to compare them nor to create yet another procedure for a particular problem. Rather it was to provide users with a new integrative tool conceived to grow modularly offering common features and different algorithms (two at the moment) with user friendly options, metadata filtering, short pre-processing time and enough flexibility to be used on different ecological cases. A significant feature of APHIS that distinguished it from most of the existing software is that pictures can be processed in batches because the pre-processing and the photo-matching phases are independent. The software was designed to pack unsupervised and supervised tasks into separate working phases, allowing the sequential treatment of pictures in a batch. The photo-matching is an automated task that requires long computation times and does not need to be monitored. Such work-flow should limit the time invested by the

user to the pre-processing and the post-processing phases only. This minimizes the time spent by the user in front of the computer.

This is a simple but relevant improvement in front of solutions where automated photo-matching should be started and inspected independently for every picture. Another novel aspect is that APHIS creates log files that can be used to track the analyses and kept for successive examinations. A companion procedure ('Results Digest', freely available at http://www.imedea.uib-csic.es/bc/ ecopob/) was built to extract results from the log files generated by APHIS. It generates a table by ordering images of each individual according to date. Users can obtain the photo-history of each individual for further analysis and any image can be inspected by simply selecting its name from the table. At present APHIS allows users to choose between two matching approaches, the Spot Pattern Matching (SPM) and the Image Template Matching (ITM). In the SPM approach, based on the algorithm of the existing I3S software (Van Tienhoven et al., 2007), the comparison between sample and candidate pictures is made only across pairs of spots marked at both pictures. This reduces differences among patterns and focuses the analysis on common or easily identified body marks. The SPM approach tolerates some discrepancies in the image rotation angle between sample and candidate pictures (Speed et al., 2007). This is possible because of the affine spatial correction applied, which simulates a comparison between spot patterns in the same two-dimensional space. Although this method unrealistically assumes that the individual is two-dimensional, it can be considered a good approximation if the region of interest is selected at a flat and rigid part of the individual's body (Van Tienhoven et al., 2007). The major drawback of the SPM is that pre-processing time is long as far as the user has to manually select 20–50 spots on each photograph to create the image 'fingerprint'. However, this method correctly identified 100% of recaptures when applied to the lizard dataset. Another remark is that the metadata based filtering system provided for

SPM can reduce the computing time but an erroneously assigned value can prevent the detection of a real match. In contrast, the ITM approach reduces the pre-processing effort to only the setting of two reference spots per picture that will be used for rotation as well as processing starting points (Fig. 3). However, it is important to set uniquely recognizable body parts or marks as reference points because the reliability of matching scores is extremely dependent of the reproducibility of this selection across samples. In this case, we decided to use the normalized versions of the template matching algorithm to minimize the effect of differences in brightness between templates in calculating the matching scores (Bradski, 2000). Additionally, the simultaneous use of six templates was incorporated to prevent possible distortions affecting only a specific portion of the image (Fig. 3). Despite these efforts to optimize the robustness of the method, the study of lizards shows that ITM keeps being especially sensitive to luminosity differences from sample to sample, local bright spots or shadows, the angle between the individual and the photographic axis or the body torsion. These drawbacks can affect the score values and mask real matches, although with very contrasted and defined chromatic patterns, as in the case of the spectacled salamander, the procedure succeeded in finding matches among images of very different quality (Fig. 5). The need to assume that the individual is two-dimensional also limits this method.

However, if photo characteristics can be kept similar across samples or if the patterns are highly contrasted the ITM becomes a fast and versatile analytical approach to be used for comparing almost any visually identifiable natural mark. Another aspect of ITM is that the pre-processing is limited to the set-up of reference points. This simplifies the standardization of manual annotation and facilitates the use to non-experienced users. Also ecological studies where matching accuracy is not critical, i.e. age determination based in the observation of progressively changing patterns in skin, feather or fur pigmentation, could

benefit from approaches minimizing user intervention. In conclusion, the SPMis a highly precise photo-identification method for species showing variable patterns of lepidosis (see also Sacchi et al., 2010) and resulted in no identification errors. The ITM approach is recommended when marks are clearly visible, i.e. highly contrasted with the background color, and/or light conditions similar across pictures while SPM is optimal when photo conditions cannot be standardized, i.e. animals are not always in the same position or the exposure changes across pictures. Strengths of both approaches are summarized in Table 2, providing basic guidelines to researchers deciding which approach could better suit their studies.

	Need of visible marks	Tolerance	Pre-processing effort	Matching efficiency	Versatility
SPM	Low	High	High	High	Moderate
ITM	Moderate to high	Low	Low	Moderate to high	High

Table 2. Comparisons between the two photo-matching approaches currently available in APHIS. 'Tolerance' is the tolerance to brightness, image definition and body deformations; 'Versatility' is the possibility to customize the parameters used by the matching routine.

Finally, the two procedures available in APHIS are semi-manual and images need to be pre-processed by the user before the matching routine begins. Although the pre-processing phase is fast and not demanding (especially in ITM), there are photo-matching procedures, such as training algorithms for facial or shape recognition, for example, that do not need pre-processing (Journaux et al., 2008; Smach et al., 2007). However these procedures typically use multiple images from different angles or with different luminosity to train the algorithms. In many ecological studies, like ours, only one image is taken for each individual and shooting multiple pictures would increase animal handling time. In addition in a semi-manual procedure the process is interactive and users decide which features of the image have to be matched or

discarded (Van Tienhoven et al., 2007). For this reason, most available procedures for animal photo matching (see a list above) are semi-manual.

Further development

APHIS aims to provide a 'suite' to incorporate different photo-matching routines so that users would choose the most appropriate one. At present APHIS include two alternative approaches, the SPM and the ITM. A natural future advance would be to include other approaches as those recently developed on the Scale Invariant Feature Transform (SIFT, Lowe, 2004). The SIFT is a computer vision approach that has been proposed by several authors as suitable for photo-identification ecological studies (Buonantony, 2008; Yu et al., 2013). The approach has shown to perform a reliable matching between images of the same object, being robust in front of scale, rotation, affine, 3D viewpoint, noise and illumination differences (Lowe, 2004). Examples of software with a SIFT-based routine are Wild-ID (Bolger et al., 2012) and Manta Matcher (Town et al., 2013). APHIS offers a metadata filtering mechanism (Fig. 2), which is not present, as far as we know, on most of the available photo-matching software. Also the ITM includes an interesting characteristic that is to divide the pattern into six sub-patterns that are analyzed independently and contribute partially to the final score. A measure that minimizes the effect of local distortions. Finally, it will be interesting to include an initial procedure to assist users in choosing the best routine available for the case considered.

Acknowledgments

We thank all the people that have helped in collecting animal images. In particular, Marco Basile, Andrea Costa, Aldo Crisci, Daniele Scinti-Roger, Mario Posillico, Rodolfo Bucci, Filippo Della Civita and all members of the Population Ecology Group at the IMEDEA. The Northern spectacled salamander project was supported by the MANFOR CBD project (LIFE09 ENV/IT/000078).We are grateful to L. Bonnet for her help with logistics at

Moltona island. Capture permits of *S. perspicillata* were obtained from the Italian Ministry of Environment (PNM-II-2012-0015691). The permission to manipulate Balearic lizards was granted by the "Conselleria de Medi Ambient" of the Government of the Balearic Islands. Funds for the Balearic Lizard project were provided by the Spanish Minister of Education and Innovation (project: BFU2009-09359) and by the Regional Government of Balearic Islands and FEDER (Ref. 48/11 "Grupo de Ecología de Poblaciones").

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APPENDIX

Ecological Informatics 27 (2015) 64–70

Contents lists available at ScienceDirect

Ecological Informatics

journal homepage: www.elsevier.com/locate/ecolinf

APHIS: A new software for photo-matching in ecological studies

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ARTICLE INFO

Article history:
Received 1 October 2013
Received in revised form 13 March 2015
Accepted 18 March 2015
Available online 25 March 2015

Keywords:
Photo-identification
Capture–recapture
Marking
Individual-based data

ABSTRACT

Unique body characteristics are increasingly used for individual recognition to avoid the effort and the potential negative effects of capture–mark–recapture technique. As a consequence there is a growing demand for computer procedures to assist users in photo-recognition of an individual. We present a new software for photo-matching developed to minimize the pre-processing time and maximize the speed of the matching procedure. In APHIS photos can be processed in batches of hundreds and users can select between two alternative matching procedures, one interactive, built as an extension of existing and freely available software, and one automatic. We assessed its performance in terms of individual recognition and time efficiency and illustrate its use with real capture-photo–recapture studies on a reptile and an amphibian species, the Balearic Lizard *Podiarcis lilfordi* and the Northern spectacled salamander *Salamandrina perspicillata*, with contrasting skin patterns.

1. Introduction

Detailed data on individual life-history are used in ecological and evolutionary studies for the estimate of demographic parameters such as population size, survival and fertility of wildlife populations (e.g. Fernández-Chacón et al., 2011; Lebreton and North, 1993; Tavecchia et al., 2001, 2005; Williams et al., 2001). A common solution for the individual recognition of the animals is to apply a mark to the animal body in the form of a tag or a ring with a unique alphanumeric code. However, rings, tags, flipper bands or other marks can alter individual fates and behavior (Gauthier-Clerc et al., 2004; McCarthy and Parris, 2004). In addition to ethical issues (e.g. May, 2004), these negative effects lead to bias the estimates of the parameters of interest. As a consequence there is an increasing interest in using non-invasive methods for individual recognition, such as unique natural marks or body characteristics. These methods have been applied with success in a wide range of taxa, in mammals (Karanth and Nichols, 1998; Langtimm et al., 2004; Martínez-Jauregui et al., 2012), amphibians (Gamble et al., 2008), reptiles (Sacchi et al., 2010), fishes (Speed et al., 2007; Van Tienhoven et al., 2007) or cephalopods (Huffard et al., 2008). However, with few exceptions (i.e. Perera et al., 2001), the photo-identification is restricted to those species featuring distinct colors, spots or marks. Photo-identification procedures consist of comparing a sample picture of an unknown individual with a library of candidate images of previously photographed individuals. This search is, in many cases, conducted by experienced observers who compare patterns and scars between photographs with the naked eye and might be extremely time-consuming when library contains hundreds of images (e.g. Martínez-Jauregui et al., 2012; Verborgh et al., 2009). Naked-eye comparisons are typically assisted by a preliminary grouping of the images using a multi-character score, for example by grouping images with a given chromatic pattern (e.g. absence or presence of specific marks, Carafa and Biondi, 2004). Unaided procedures may also become prone to errors when image libraries expand. There is now a growing demand in developing automatic or computer-aided procedures for photo-matching (Gamble et al., 2008). A computer-aided photo-identification system identifies the most probable sample–candidate matches, reducing the number of images to be inspected. Most photo-identification software solutions concatenate three processing steps. The first is a preprocessing step where a region of interest is selected and the image rotated, scaled or spatially corrected if required by comparison algorithms; the second is usually an automated comparison between the sample and the library of images, which arranges candidates by matching probability or likelihood values; a final step is a visual comparison of sample–candidate pairs for a limited number of plausible matches.

We present a new software solution, APHIS (Automated PHoto-Identification Suite), specially designed to deal with sample sets of over a hundred photographs per field campaign and image libraries containing more than a thousand samples. APHIS proposes two approaches for photo-matching, the Spot Pattern Matching (SPM) and

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http://dx.doi.org/10.1016/j.ecoinf.2015.03.003
1574-9541/

the Image Template Matching (ITM). The former has been built on the already existing I^{3}S algorithm (Van Tienhoven et al., 2007) while the latter is a novel approach based on pixel matching that minimizes the user's preprocessing effort. ITM is a fast-running alternative to study species with apparent or easily recognizable spots or colored parts of the skin. The workflow and graphic interface of APHIS have been designed to reduce the time invested by the researcher in analytical tasks and to enhance user experience. We describe below the general features of the APHIS interface and illustrate the SPM and ITM procedures using real data from two capture-photo–recapture studies on the Balearic Lizard, *Podarcis lilfordi*, and on the Northern spectacled salamander, *Salamandrina perspicillata* (Fig. 1).

2. Material and methods

2.1. Automated PHoto-Identification Suite (APHIS)

APHIS (Automated PHoto-Identification Suite, freely available at http://www.imedea.uib-csic.es/bc/ecopob/) v. 1.0 combines C++ and Java modules. The idea behind APHIS was to provide users with a flexible environment for photo handling and matching. The Graphic User Interface (GUI) has been programmed using the Nokia Qt framework (http://qt.nokia.com/). The image preprocessing and analysis of the ITM approach implements functions from the openCV v. 2.2 libraries (Bradski, 2000). The two available approaches, SPM and ITM, differ in how they treat and match the sample pictures. They perform differently depending on photo and species characteristics (see below). APHIS also implements a metadata based filtering system for its SPM approach, a feature present in other photo-identification software solutions, i.e. Manta 2.1 and Contour 3.0 versions from the I^{3}S series (http://www.reijns.com/i3s/). This function allows the user to predefine species-specific descriptive features and their possible alternative values for characterizing each sample. For example, a commonly useful feature would be the sex of the individual. A filtered search will only be conducted among sample–candidate pairs having equivalent character values and will substantially reduce the photo-matching time.

Finally, an important feature in APHIS is the automatic creation of log files that register the score lists obtained at each comparison. It also produces a registry of the matches validated by the user, which will lead to an easy analysis of capture–recapture data.

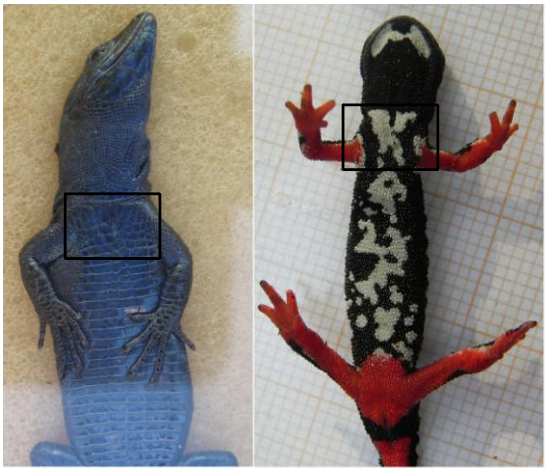

Fig. 1. The ventral side of a Balearic lizard (left) and of a Northern spectacled salamander (right). The black rectangles mark the region used for individual photo-recognition. (For interpretation of the references to color in this figure, the reader is referred to the web version of this article.)

2.2. The Spot Pattern Matching approach (SPM)

The Spot Pattern Matching approach is based on the algorithm implemented in the freely available software I^{3}S (Van Tienhoven et al., 2007). Each sample picture is pre-processed by the user before the photo-matching. During pre-processing the user delimits the region of interest with a given number of unequivocally identifiable reference points (typically three) and marks the set of spots within that will be compared during the matching of the defined area (Fig. 2; see Sacchi et al., 2010; Speed et al., 2007; Van Tienhoven et al., 2007 for practical examples). The coordinates of each spot in the space delimited by the reference points form a fingerprint-like statistic. At the matching step, the spatially-corrected cloud of spots from the sample is compared with the fingerprints stored in a repository. This correction is the result of an affine transformation of the sample pattern mapped onto that of candidate one. Matching scores are calculated as the sum of metric distances between spots from every pair created in a sample–candidate comparison divided by the square of the total number of spot pairs. Lowest scores point to likely matches, being the number of spot pairs used during calculation relevant to the resulting score value (Fig. 2), although it is not yet clear as to what extent (Speed et al., 2007). Sacchi et al. (2010) used a range of 20 to 40 spots per image and found a negative association between the matching score and the number of points, but this effect was not large enough to impair matching results. The matching algorithm used by SPM approach in APHIS was directly extracted from the I^{3}S Classic source code in accordance with its developers respecting its license agreement (GNU Public License v2). APHIS uses the exhaustive search version of the comparison algorithm described at Van Tienhoven et al. (2007). The exhaustive search uses every possible three spot pairs as reference points for different affine transformations, and not only those defined by the user (quick search). Score values are calculated for each transformation in comparison with the candidate and the lowest score is kept as final result. Exhaustive searches, although computer resource consuming, proved to be far more accurate than simple ('quick') searches (Van Tienhoven et al., 2007). Differently from the I^{3}S software, the pre-processing and the matching phases in APHIS occur separately. This permits to process sequentially a group of samples and then launch the matching calculations for the whole set. Once the automated matching is finished, the user is presented with a list of sample–candidate alternatives ordered from lower to higher matching scores (Fig. 3). If multiple pictures from the same candidate are available, APHIS only shows these with the lowest score. Finally, the user should inspect the possible candidates and accept the candidate as a recapture or discard the matching and register the sample as a new individual in the repository.

2.3. The Image Template Matching (ITM) approach

The Image Template Matching approach has been conceived to minimize the time invested by the user at the pre-processing step. It implements the *matchTemplate* function of the Open Computer Vision libraries (OpenCV, Bradski, 2000), a preprogrammed function that slides a template image patch over an input image looking for matches. This method provides three different algorithms and their normalized versions in order to calculate a matrix of likelihoods of match per comparison. APHIS implements the normalized version of the correlation coefficient algorithm, which is the most accurate of the three (Bradski, 2000). Normalization is recommended to minimize the effect of lightning differences among template and input while calculating matching scores (Bradski, 2000).

During the ITM pre-processing step the user selects only two reference points for each picture (Fig. 4). It is extremely important to use small, spot-like and easily recognizable parts or species-characteristic natural marks as reference points. The reliability of matching scores will depend on the reproducibility of this selection across pictures. APHIS automatically transforms to gray scale, rotates and resizes the

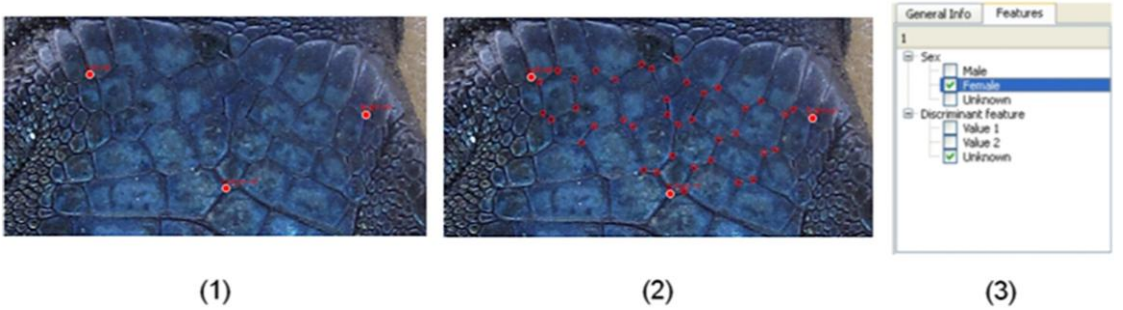

Fig. 2. Preprocessing for the SPM approach is divided into two steps: (1) manual selection of three reference points and (2) manual selection of between 30 and 50 intersections. Optionally, a third step (3) can be applied if the user decides to use individual discriminant characters to reduce processing times and to improve the quality of the resulting candidate list by constraining the analysis within individuals that show a given character.

images aligning the reference points along a horizontal axis. Next, a pattern, which is the region containing the natural marks used for identification, is cropped from the sample images (Fig. 4). The result is a rectangular area delimited by the pixel distance between reference points as base and a height 105% of this distance. The rectangle bottom is placed with a number of pixels below the reference points equal to 10% of the horizontal distance between them. A scale factor is applied to the resulting images such that all patterns finish aligned by their reference points and with a fixed resolution of 460×436 pixels. Finally, six templates of 91×103 pixels are homogeneously cut out from the pattern, distributed in two non-overlapping rows and three non-overlapping columns (Fig. 4). Using six non-overlapping templates the effect of local image defects has less impact on the final score, enabling real matches to be well positioned in the score list ahead of random

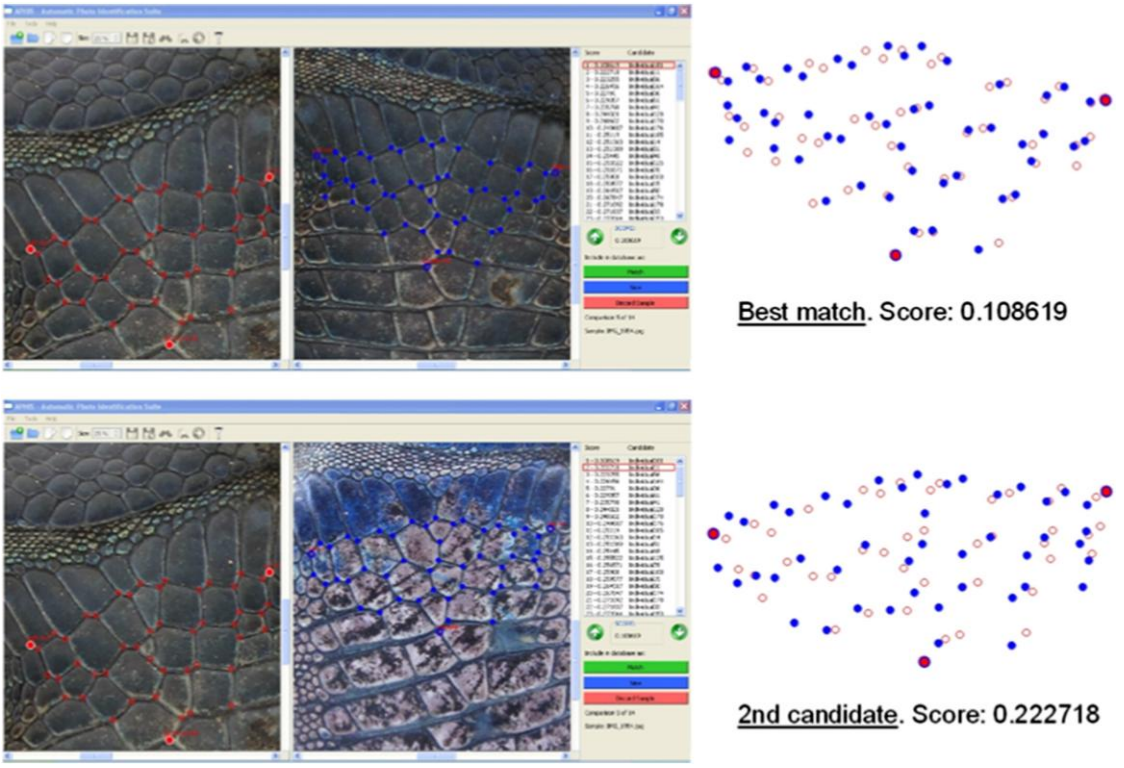

Fig. 3. The affine transformation of the fingerprint happens during the matching step and is comparison-dependent as far as it is applied for each sample–candidate pair under analysis. This figure shows the superposition of sample–candidate fingerprints for the first (best match) and second candidates of a SPM comparison from our study. It also includes two screen-shots of the APHIS display during the visual inspection of both comparisons. The sample is situated to the left of the screen, the candidate in the middle part and the controls used for decision-making to the right. The area reserved to display images has been maximized to show a general view of the animal that can be of help during the identification.

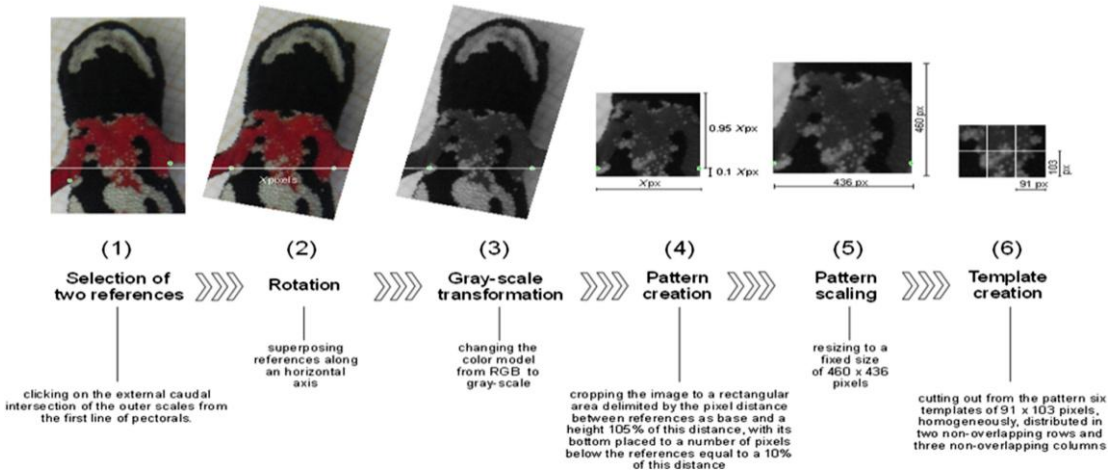

Fig. 4. Preprocessing for the ITM approach is divided into six steps: (1) manual selection of two references, (2) references are aligned with the horizontal axis, (3) the RGB image is transformed to gray-scale, (4) a region of interest is selected, (5) the image is resized to a fixed resolution, the resulting image is the pattern and (6) six non-overlapping contiguous templates are extracted automatically from the lower half of the pattern. Steps (2) to (6) are automatically performed by the software and do not need supervision.

ones. It has to be noted that values used to delimit patterns and templates are not arbitrary; they were expressly set to delimit and subdivide the region of the animal that includes the natural marks. In the case of the common wall lizard, *Podarcis muralis*, for example, this region is the pectoral area which includes a scales pattern characteristic for each individual (Fig. 1; Sacchi et al., 2010). Note that the rectangular area is fixed in the current version of APHIS (460 × 436 pixels) to fulfill the needs of the current studies; however it can be set to any arbitrary value. At present this can be done only by changing the source code (line 12–16 of the source code 'ITM.cpp') but further development of APHIS will make it possible to set the area directly using the GUI. The six resulting templates extracted from a single sample are individually compared with the candidate pattern and the scores resulting from the comparisons are added up to produce the final sample–candidate matching score. APHIS produces an ITM score list per comparison where candidates are ordered, from highest to lowest, by their matching likelihood (the final score). Individual template scores range from −1 to 1, this being the score obtained when the template is a portion of the own input image. Therefore a value of 6 would be a perfect sample–candidate match.

2.4. The capture-photo–recapture studies

We used real data from a capture-photo–recapture study on the Balearic lizard and on the Northern spectacled salamander to assess software matching performance in terms of individual recognition and time efficiency. Both studies aimed to estimate survival and population size using longitudinal data collected during multiple capture-photo–recapture sessions (e.g. Ruiz de Infante Antón et al., 2013; Tenan et al., 2013; Williams et al., 2001). The ventral region of both species is highly variable and preliminary studies have shown that the ventral patterns can be sued for individual recognition (Carafa and Biondi, 2004; Perera et al., 2001). In the Balearic lizard (dark morph) the ventral region is characterized by a uniform dark-blue or dark-gray color (Fig. 1) and individuals differ in the position and dimension of their ventral scales. The ventral region of the spectacled salamander has white, black and red areas of variable shapes and dimensions (Fig. 1) with marked differences across individuals in the color patterns.

Lizards were captured at the island of Moltona off the southern coast of Mallorca (Balearic archipelago, Spain) for three consecutive days in two sessions, June and October 2010 with pit fall traps positioned along and inside shrubs within an area of c. 0.21 ha (Ruiz de Infante Antón et al., 2013; Tenan et al., 2013). Captured individuals were held under a glass to ensure a clear picture of their ventral scales (Figs. 1, 2, 3 and 5). Photos were taken using a digital camera (Canon© G10) fixed to a stand and positioned inside a photo-cube to standardize light conditions. The picture was made after aligning lens marks with the collar of the individual to diminish differences in rotation, translation or lighting across the pictures. After manipulation, lizards were released. To assess the performance of the photo-identification method, all individuals were double-marked using a low-temperature medical cauterizing unit (Winne et al., 2006). Images of the Northern spectacled salamander have been collected in an area of c. 1 ha of the "Monte di Mezzo" Natural Reserve as a part of a large-scale ecological study (MANFOR CBD; LIFE09 ENV/IT/000078). Animals were captured by hand during their terrestrial activity in two sessions of two consecutive days twenty days apart (8–9 and 28–29 October 2013). Images were taken at low resolution (1280 × 960 pixel) using a digital camera (Nikon© Coolpix P100), at variable distance from the subject and without standardizing light conditions. Individuals were first identified by assigning a binary code to each image on the basis of four chromatic characters as suggested in Carafa and Biondi (2004). These results were used to assess the percentage of correctly identified matches by APHIS. Given the belly pattern of salamanders and the absence of clearly identifiable spot-like points, the ventral images were processed using ITM approach, only.

In each study the images taken the first day were used to create the initial repository. APHIS classified each subsequent photo as a recapture or as a new individual whether a match was found in the existing catalog or not, respectively. When a match is found the processed image is stored in the same directory of the matched sample, otherwise a new directory is created. Each processed image is considered as candidate for next comparisons, so that multiple images from the same individual taken in different capture-photo–recapture sessions are treated as independent samples. The reliability of both approaches implemented in APHIS was assessed by recording the number of correctly classified recaptures. For each misclassified picture we assessed the phase in which it occurred and inspected photo characteristics to identify possible physical character responsible for the misclassification. In addition to the real sets of image, to evaluate time efficiency of the SPM and ITM

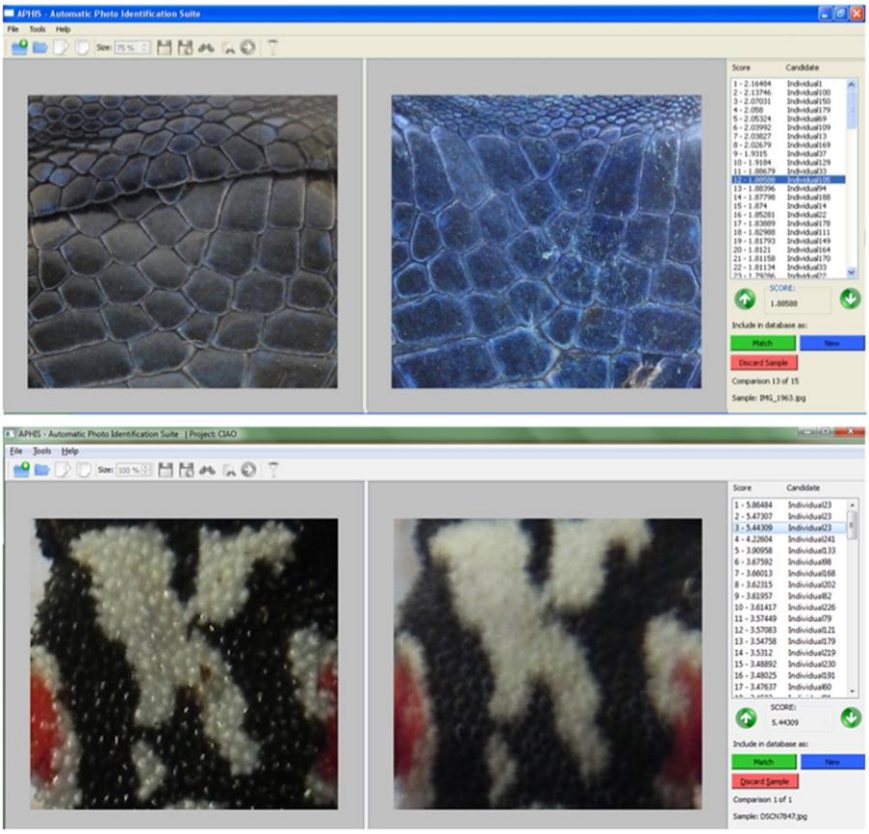

Fig. 5. Matching between a sample image (left) and each of the candidates (right), classified according to the match score (e.g. the likelihood of a correct match). When the pattern to match is uniform, ITM is sensitive to image luminosity, local bright spots or shadows and body torsion. The upper photo shows a correct match that was classified at position twelve in the list of candidates, probably due to the difference in brightness between the sample and the candidate images. However, when the pattern to match is highly contrasted ITM has proved to be less sensitive to image quality. The lower figure shows a match classified among the first three even when the library image (right) was blurred.

approaches we randomly choose 100 samples of Balearic lizard and analyzed them against a repository of a thousand candidates in a computer with an Intel Core Duo 2.40 GHz processor with 3 GB of RAM memory. In general, SPM approach required between 30 and 50 points per individual and, in both approaches the 20 first candidate pictures proposed by APHIS as potential matches were inspected before assignment. We have calculated the total time invested by the user in supervised tasks of this analysis, which include the pre-processing steps (i.e. the marking of scale intersections in SPM approach) and the visual comparison of likely matches after the processing phase occurred.

3. Results

The 287 images, of which 91 were recaptures, were analyzed using the SPM and the ITM procedures. The two approaches, ITM and SPM, delivered similar results, however, the overall number of photos classified as new captures by both approaches were different. The SPM approach correctly classified all newly photographed individuals (percentage of correctly classified pictures = 100%), while ITM found 85 of the 91 recaptures (93.4%). Excluding user's mistakes (e.g. reference points placed wrongly), ITM approach resulted in 95.6% of correctly classified recaptures. The three errors were due to image characteristics such as marked differences in luminosity, local shadows, or variations in pigmentation or lepidosis, i.e. the scale pattern deformation due to body torsion. Nineteen of 305 images of northern spectacled salamander were recaptures. In this case the ITM approach correctly classified them all (100%). The whole ITM analysis took 52 min against the 215 min of the non-automatized method originally used to determine the number of recaptures. The analysis of 100 recaptures on a repository of a thousand took a total of 329 min with ITM and 266 min with SPM (Table 1), done in separate intensive batch sessions. However, the time invested in supervised tasks for the ITM approach (104 min) was nearly a half of this invested at the SPM approach (197 min; Table 1). The unsupervised task took 225 min for ITM and 69 min for SPM.

Table 1
Time spent in photo-matching of lizard images using a batch search of 100 samples against a repository of 1000 individuals. Supervised tasks include pre-processing (reference points and pattern edition) and post-processing (visual inspection of likely matches); the calculation of scores for each sample–candidate pair is automatically conducted by the software, therefore it is an unsupervised task.

Approach	Time (min.)		
	Total	Supervised tasks	Unsupervised task
SPM	266	197	69
ITM	329	104	225

Hence the ITM approach had a longer processing time but the pre-processing phase was faster. On the other hand the longer pre-processing phase in SPM was paid off by a short processing period.

4. Discussion

Individual identification by photo recognition is becoming an increasing area of research. At present, there are several routines available for photo-matching, for example 'I^{3}S' (van Tienhoven et al., 2007),'MantaMatcher' (Town et al., 2013), 'StripeSpotter' (Lahiri et al., 2011), Sloop (Gamble et al., 2008) and 'Wild-ID' (Bolger et al., 2012). Some are highly customized and some are very flexible. Our purpose here was neither to compare them nor to create yet another procedure for a particular problem. Rather it was to provide users with a new integrative tool conceived to grow modularly offering common features and different algorithms (two at the moment) with user friendly options, metadata filtering, short pre-processing time and enough flexibility to be used on different ecological cases. A significant feature of APHIS that distinguished it from most of the existing software is that pictures can be processed in batches because the pre-processing and the photo-matching phases are independent. The software was designed to pack unsupervised and supervised tasks into separate working phases, allowing the sequential treatment of pictures in a batch. The photo-matching is an automated task that requires long computation times and does not need to be monitored. Such work-flow should limit the time invested by the user to the pre-processing and the post-processing phases only. This minimizes the time spent by the user in front of the computer. This is a simple but relevant improvement in front of solutions where automated photo-matching should be started and inspected independently for every picture. Another novel aspect is that APHIS creates log files that can be used to track the analyses and kept for successive examinations. A companion procedure ('ResultsDigest', freely available at http://www.imedea.uib-csic.es/bc/ecopob/) was built to extract results from the log files generated by APHIS. It generates a table by ordering images of each individual according to date. Users can obtain the photo-history of each individual for further analysis and any image can be inspected by simply selecting its name from the table. At present APHIS allows users to choose between two matching approaches, the Spot Pattern Matching (SPM) and the Image Template Matching (ITM). In the SPM approach, based on the algorithm of the existing I^{3}S software (Van Tienhoven et al., 2007), the comparison between sample and candidate pictures is made only across pairs of spots marked at both pictures. This reduces differences among patterns and focuses the analysis on common or easily identified body marks. The SPM approach tolerates some discrepancies in the image rotation angle between sample and candidate pictures (Speed et al., 2007). This is possible because of the affine spatial correction applied, which simulates a comparison between spot patterns in the same two-dimensional space. Although this method unrealistically assumes that the individual is two-dimensional, it can be considered a good approximation if the region of interest is selected at a flat and rigid part of the individual's body (Van Tienhoven et al., 2007). The major drawback of the SPM is that pre-processing time is long as far as the user has to manually select 20–50 spots on each photograph to create the image 'fingerprint'. However, this method correctly identified 100% of recaptures when applied to the lizard dataset. Another remark is that the metadata based filtering system provided for SPM can reduce the computing time but an erroneously assigned value can prevent the detection of a real match. In contrast, the ITM approach reduces the pre-processing effort to only the setting of two reference spots per picture that will be used for rotation as well as processing starting points (Fig. 3). However, it is important to set uniquely recognizable body parts or marks as reference points because the reliability of matching scores is extremely dependent of the reproducibility of this selection across samples. In this case, we decided to use the normalized versions of the template matching algorithm to minimize the effect of differences in brightness between templates in calculating the matching scores (Bradski, 2000). Additionally, the simultaneous use of six templates was incorporated to prevent possible distortions affecting only a specific portion of the image (Fig. 3). Despite these efforts to optimize the robustness of the method, the study of lizards shows that ITM keeps being especially sensitive to luminosity differences from sample to sample, local bright spots or shadows, the angle between the individual and the photographic axis or the body torsion. These drawbacks can affect the score values and mask real matches, although with very contrasted and defined chromatic patterns, as in the case of the spectacled salamander, the procedure succeeded in finding matches among images of very different quality (Fig. 5). The need to assume that the individual is two-dimensional also limits this method. However, if photo characteristics can be kept similar across samples or if the patterns are highly contrasted the ITM becomes a fast and versatile analytical approach to be used for comparing almost any visually identifiable natural mark. Another aspect of ITM is that the pre-processing is limited to the set-up of reference points. This simplifies the standardization of manual annotation and facilitates the use to non-experienced users. Also ecological studies where matching accuracy is not critical, i.e. age determination based in the observation of progressively changing patterns in skin, feather or fur pigmentation, could benefit from approaches minimizing user intervention. In conclusion, the SPM is a highly precise photo-identification method for species showing variable patterns of lepidosis (see also Sacchi et al., 2010) and resulted in no identification errors. The ITM approach is recommended when marks are clearly visible, i.e. highly contrasted with the background color, and/or light conditions similar across pictures while SPM is optimal when photo conditions cannot be standardized, i.e. animals are not always in the same position or the exposure changes across pictures. Strengths of both approaches are summarized in Table 2, providing basic guidelines to researchers deciding which approach could better suit their studies.

Finally, the two procedures available in APHIS are semi-manual and images need to be pre-processed by the user before the matching routine begins. Although the pre-processing phase is fast and not demanding (especially in ITM), there are photo-matching procedures, such as training algorithms for facial or shape recognition, for example, that do not need pre-processing (Journaux et al., 2008; Smach et al., 2007). However these procedures typically use multiple images from different angles or with different luminosity to train the algorithms. In many ecological studies, like ours, only one image is taken for each individual and shooting multiple pictures would increase animal handling time. In addition in a semi-manual procedure the process is interactive and users decide which features of the image have to be matched or discarded (Van Tienhoven et al., 2007). For this reason, most available procedures for animal photo-matching (see a list above) are semi-manual.

4.1. Further development

APHIS aims to provide a 'suite' to incorporate different photo-matching routines so that users would choose the most appropriate one. At present APHIS include two alternative approaches, the SPM

Table 2
Comparisons between the two photo-matching approaches currently available in APHIS. 'Tolerance' is the tolerance to brightness, image definition and body deformations; 'Versatility' is the possibility to customize the parameters used by the matching routine.

	Need of visible marks	Tolerance	Pre-processing effort	Matching efficiency	Versatility
SPM	Low	High	High	High	Moderate
ITM	Moderate to high	Low	Low	Moderate to high	High

and the ITM. A natural future advance would be to include other approaches as those recently developed on the Scale Invariant Feature Transform (SIFT, Lowe, 2004). The SIFT is a computer vision approach that has been proposed by several authors as suitable for photo-identification ecological studies (Buonantony, 2008; Yu et al., 2013). The approach has shown to perform a reliable matching between images of the same object, being robust in front of scale, rotation, affine, 3D viewpoint, noise and illumination differences (Lowe, 2004). Examples of software with a SIFT-based routine are Wild-ID (Bolger et al., 2012) and Manta Matcher (Town et al., 2013). APHIS offers a metadata filtering mechanism (Fig. 2), which is not present, as far as we know, on most of the available photo-matching software. Also the ITM includes an interesting characteristic that is to divide the pattern into six sub-patterns that are analyzed independently and contribute partially to the final score. A measure that minimizes the effect of local distortions. Finally, it will be interesting to include an initial procedure to assist users in choosing the best routine available for the case considered.

Acknowledgments

We thank all the people that have helped in collecting animal images. In particular, Marco Basile, Andrea Costa, Aldo Crisci, Daniele Scinti-Roger, Mario Posillico, Rodolfo Bucci, Filippo Della Civita and all members of the Population Ecology Group at the IMEDEA. The Northern spectacled salamander project was supported by the MANFOR CBD project (LIFE09 ENV/IT/000078). We are grateful to L. Bonnet for her help with logistics at Moltona island.

Capture permits of *S. perspicillata* were obtained from the Italian Ministry of Environment (PNM-II-2012-0015691). The permission to manipulate Balearic lizards was granted by the "Conselleria de Medi Ambient" of the Government of the Balearic Islands. Funds for the Balearic Lizard project were provided by the Spanish Minister of Education and Innovation (project: BFU2009-09359) and by the Regional Government of Balearic Islands and FEDER (Ref. 48/11 "Grupo de Ecología de Poblaciones") .

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Chapter I.2

Estimating lizard population density: An empirical comparison between line-transect and capture-recapture methods.

Jon Ruíz de Infante, **Andreu Rotger**, José Manuel Igual and Giacomo Tavecchia (2014). Wildlife Research 40, 552-560.

Estimating lizard population density: An empirical comparison between line-transect and capture-recapture methods.

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Abstract

Context: In most natural populations, exhaustive counts are not possible and estimates need to be derived from partial sampling by using analytical methods that account for biological processes, sampling errors and detection probability. The methods available have contrasting pitfalls and payoffs in relation to the assumptions made but are seldom contrasted on the same population.
Aims: We compared density estimates derived by different sampling methods. Despite the real density being unknown, the 'soft' validation of density estimates might help to better understand the possible pitfalls and payoffs of each method. This was done in three closed populations and with three different habitat typologies to disentangle the effects of different capture-detection processes to those introduced by the method itself.
Methods: We considered the problem of estimating population density of the endemic Balearic lizard, *Podarcis lilfordi*, in three island populations. We compared estimates derived by distance sampling (LT) in three types of habitat with those calculated from a simultaneous 3-day capture–mark–recapture study. Capture histories of marked individuals were used to estimate density using spatially explicit capture–recapture models (SECR) and a capture–mark–recapture model without spatial data (CMR). Moreover, we empirically assessed the influence of survey duration by extending the survey in the largest island to five occasions. The real

population density was unknown and absolute accuracy of each method cannot be assessed; nevertheless, relative estimates might be informative.
Key results: LT estimates had the greatest coefficient of variation in vegetated habitats, corresponding to possible departures from model assumptions. SECR estimates differed among islands and were from 12% to 37% lower than those derived by LT but only in the largest islands with high and dense vegetation. CMR estimates depended on the number of occasions whereas SECR did not and showed lower variance. LT and SECR estimates showed differences across islets.
Conclusions: Line-transect and capture–recapture methods gave comparable results but the interaction between recapture processes and habitat types should be considered when inferring density to the whole area. We found density estimates between 1500 and 2500 individuals ha–1, being a higher value than those found for lizards in continental regions.

Additional keywords: detection, distance sampling, habitat, island, SECR.

Resum

En la majoria de les poblacions naturals els recomptes exhaustius no són possibles i les estimacions s'han d'obtenir directament de mostrejos parcials mitjançant l'ús de mètodes analítics en resposta dels processos biològics, errors de mostreig i probabilitat de detecció. Els mètodes disponibles mostren diferents dificultats i desavantatges en relació amb les suposicions fetes, però poques vegades són contrastades en la mateixa població.

Es van comparar els valors de les densitats obtingudes per diferents mètodes de mostreig. Tot i que la densitat real és desconeguda, la validació "acurada" de les estimacions de densitat podria ajudar a entendre millor els possibles desavantatges i beneficis de cada mètode. Aquest procés es va realitzar en tres poblacions tancades i amb tres diferents tipologies d'hàbitat per així diferenciar els efectes dels diferents processos de captura i de detecció dels introduïts pel mateix mètode.

Es va considerar el problema d'estimar la densitat de població de la sargantana endèmica de les Balears, *Podarcis lilfordi*, en tres poblacions de tres illes diferents. Es van comparar les estimacions obtingudes pel mostreig de distàncies per transsectes (LT) en tres tipus d'hàbitat calculats a partir d'un estudi de 3 dies de captura-marcatge-recaptura simultània. Les històries de captura dels individus marcats van ser utilitzades per estimar la densitat utilitzant models espacialment explícits de captura-recaptura (SECR) i un model de captura-marcatge-recaptura sense dades espacials (CMR). D'altra banda, es va avaluar empíricament la influència de la durada de l'estudi mitjançant l'ampliació d'aquest a l'illa més gran de tres a cinc ocasions. La densitat de població real era

desconeguda i la precisió absoluta de cada mètode no pot ser avaluat; malgrat això, les estimacions relatives poden ser informatives.

Les estimacions de LT van tindre el major coeficient de variació en hàbitats amb vegetació, que corresponen a possibles desviacions de les assumpcions del model. Les estimacions SECR diferien entre les illes i eren de 12% a 37% més baixes que els obtinguts per LT, però només en les illes grans amb una quantitat de vegetació alta i densa. Les estimacions CMR depenien del nombre d'ocasions mentre que SECR no ho feien i van mostrar menor variància. Les estimacions LT i SECR van mostrar diferències entre els illots.

Els mètodes per transsectes lineals i de captura-recaptura van donar resultats comparables, però la interacció entre els processos de recaptura i tipus d'hàbitat han de ser considerats quan infereixen la densitat de tota la zona. Trobem estimacions de densitat entre 1500 i 2500 individus ha-1, sent un valor superior als trobats per les sargantanes a les regions continentals.

Paraules clau: detecció, mostreig de distàncies, habitat, illes, SECR

Introduction

The distribution of individuals over space and time is a central theme in ecological theory. Yet, how to obtain a robust measure of population abundance (N), or density (D), is still an open field of research (Buckland et al. 2001; Seber 2002; Williams et al. 2002; Tavecchia et al. 2009). The problem has its root in the scientific method itself. When exhaustive counts are not possible, estimates have to be derived from partial sampling by using analytical methods that account for biological processes, sampling errors and detection probability. A first step to reduce problem complexity is to conduct surveys over a short period of time so that the population can be assumed to be closed to emigration, immigration, birth and death and only detection probability has to be considered (Otisetal.1978;Seber 2002; Williams et al. 2002). An estimate of the population density, $\widehat{D}$, definde as the number of individuals per unit area is generally preferred over an estimate of population size, $\widehat{N}$, because it allows comparisons across species and over space and time.

The two estimates are related by the area surveyed, A, so that $\widehat{D} = \widehat{N}/A$. As a consequence, population density models have typically two components, one to account for the detection process and a second to estimate A. The two sets of methods commonly used for the estimate of population density are line transect (LT) and capture–mark–recapture methods (CMR). In LT, observers count the number of animals while moving along a transect line and measure, or give an estimate of, the distance from transect line at which each individual has been seen (Buckland et al. 2001). In CMR studies, animals are captured, marked and recaptured (or resighted) and an estimate of population abundance can be derived from individual capture histories (Schwarz and Anderson 2001; Seber 2002; Williams et al. 2002). Population density can be then derived using an estimate of A, which is typically based on the relative positions of detectors (Sutherland 2006) or on animal movements, such as average home-range size (Seber 2002) or mean maximum distance moved (Wilson and Anderson 1985). These add-on methods are only partially satisfactory. Spatially explicit capture–recapture models (SECR; Efford 2004) incorporate an estimate of A with an underlying model of animal home-range based on the movement of marked animals between detectors (traps). This solution should be preferred to the conventional CMR approach because in SECR models, the estimations of $\widehat{D}$ and A occur simultaneously and density estimates or realised population size are less biased (see Borchers and Efford 2008; Noss et al. 2012; Efford and Fewster 2013 for more detailed on advantages of SECR).

At a first look, LTand capture–recapture methods differ only in the way recapture processes are treated. However, they have different pitfalls and payoffs in relation to the assumptions made and the system under study. Detection processes in LT are assumed to have the following three characteristics: (1) the detection probability decreases with the distance from the transect line, (2) individuals on the transect line are detected with certainty, and (3) individuals are detected at their initial location and their

distance from the transect line is exact (Buckland et al. 2001). Capture–recapture methods assume that recapture events are independent, that marks are not lost, that individuals are homogeneous in their capture probability and that previous captures do not influence the following encounter history. Non-spatial explicit model further assumes geographic closure, hence, the need to estimate the sampled area. SECR models do not assume geographic closure, but assume that animal home-range is approximately circular and have a fix central location (Efford 2004). In some systems, LT provides a quick and not expensive way to obtain robust estimates of population density or abundance (Aars et al. 2009). In other cases, CMR and SECR methods might be more suitable because animals are difficult to detect or LT model assumptions are not met (Hendriks et al. 2012).

We considered the problem of estimating population density of the Balearic lizard, *Podarcis lilfordi* (Squamata, Lacertidae; Günther, 1874), in three island populations. The isolated character of island populations, the reduced species diversity and the relatively small number of interactions across species alter the balance of environmental and ecological control on population dynamics (MacArthur and Wilson 1967; Wright 1980). Because of this ecological setting and the low energetic costs, lizards on islands have potential to reach exceptionally high densities following predator release, diet change and competitor release, a phenomenon referred to as 'density compensation' (MacArthur et al. 1972; Olesen and Valido 2003). Buckley and Jetz (2007) found that lizards on islands occur at densities about an order of magnitude higher than those recorded on mainland. Within the Mediterranean basin, the Balearic Islands (Spain) are one of the regions in which endemic lizards attain very dense populations (Salvador 1986; Pérez-Mellado 1989, 1998); however, the exact density value has seldom been estimated (but see Pérez-Mellado et al. 2008). Reptile populations are notoriously difficult to measure because of the small body size of the animals, secretive behaviour, habitat preferences

and fast unpredictable activity (Turner 1977). Despite these constraints, there is a long tradition of using transect sampling to estimate population densities of herpetofauna (Iverson 1978; Cassey and Ussher 1999; Germano et al. 2003; Reisinger et al. 2006; Pérez-Mellado et al. 2008). In some species of reptiles, however, individuals are difficult to detect and tend to be seen during movements, rather than before as assumed by LT models. The violation of model assumptions leads to biased estimates of density, to the point that some authors discourage the use of visual counts in estimating lizard density (Smolensky and Fitzgerald 2010). Other authors used capture–recapture methods to estimate population density and abundance (Ballinger and Congdon 1981; Kwiatkowski and Sullivan 2002); however, few have compared the two methodologies (Kacoliris et al. 2009; see also Funk et al. 2003; Smolensky and Fitzgerald 2010). Kacoliris et al.(2009) suggested that capture–recapture data might lead to less biased estimates; however, this might depend on animal behaviour and habitat characteristics (V. Pérez-Mellado, pers. comm.).

We compared lizard density estimates derived by (1)distance sampling (Buckland et al. 2001), (2) spatially explicit capture–recapture models (Efford and Fewster 2013) and (3) capture– recapture without spatial data (Seber 2002). The real density of lizards was unknown, but the 'soft' validation of density estimates (*senus* Rodda and Campbell 2002) might help better understand the possible pitfalls and payoffs of each method. We did this in three closed populations and with three different habitat typologies to disentangle the effects of different capture–detection processes to those introduced by the method itself.

Materials and methods

Species and study area

The Balearic lizard is an endemic lizard of the Balearic archipelago (Spain). It is considered endangered by the IUCN criteria (Pérez-Mellado and Martínez-Solano 2009), and vulnerable at the regional level (Viada 2006). Its distribution is currently confined to the islets of Mallorca and Menorca and to the Cabrera archipelago. We estimated lizard density in the following three islets in the south coast of Mallorca: Es Caragol (0.29ha, named 'CA' hereafter), Na Guardia (1.98ha, named 'GU' hereafter) and Na Moltona (5.09ha, named 'MO' hereafter). Vegetation richness and structure changed with islet size, with 'CA' having a low and less diverse vegetation and 'MO' with a high and more diverse one. We recognised four main habitat categories and characterised each island on the basis of their occurrence (Table1).The first habitat type, named 'A', was characterised by a vegetation higher than 50 cm, including mainly shrubs of genera *Pistacia* and *Phillyrea*, the second one, 'B', was characterised by vegetation less than 50cm (i.e. *Crithmum maritimum*), the third, 'C', comprised sand, rocks and few plants of halophytic species, such as *Salicornia ramosissima*, and the fourth, named 'D', comprised exclusively rocks and pools of salted water (Table1). These last two categories are suboptimal habitats for lizards and marked the region between the vegetated area and the sea. Nevertheless, we do not know the importance of these habitats that lizards use for shelter and in search of food.

Parameter	Es Caragol (CA)	Na Guardia (GU)	Na Moltona (MO)
Total area of the islet (ha)	0.29	1.98	5.06
High vegetation (Type A)	–	0.43	0.53
Low vegetation (Type B)	0.69		0.18
Shore (Type C)	–	0.11	0.29
Rocks (Type D)	0.31	0.46	–
Number of transects per day	3	5	7
Number of day	3	3	5
Number of traps	26	25	31
Trap array perimeter (m)	118	157	181
nTD (m)	5.9	8.1	6.3
W (m)	5.6	5.7	8.0
A(nTD) (ha)	0.125	0.233	0.270
A (ha)	0.088	0.164	0.210
Total number of lizards observed or captured			
Line transect	164	379	1507 (881[^])
Capture in grid	232	188	397 (254[^])
Recapture in grid	80	35	94 (36[^])

[^]Three days only.

Table 1. Islet area, proportions of habitat type in the islet, number of transects and characteristic of trap-arrays A = high vegetation, B = low vegetation, C = shore and scattered plants, D = rocks, nTD = nearest-neighbour distance between traps, W = average maximum distance between detections pooled over individuals, A = array area estimated by the minimum convex polygon (MCP),and A (nTD) = area of the array obtained by adding a boundary strip of constant width of 0.5 nTD. Note that Habitats A and B in GU are combined.

Line transect

In October 2012, each islet was visited during three consecutive days, with the exception of MO that was visited two additional days to evaluate the effect of a longer sampling period on density estimates. According with island surface, we considered three line transects per day at CA, five line transects per day at GU and seven line transects per day at MO, making a total of 59 line transects (Table 1). In the largest islet (MO), we were able to survey each habitat separately but this was not possible in the other two islets where transect lines would have been too short (except Habitat C in GU). Following Buckland *et al.* (2001), an observer travelled along a line of variable and known length and recorded the perpendicular distance of each observed lizard to the transect in 10-cm classes. Transects were performed daily by the same observer. We first ran a global analysis merging the data from all islets and contrasting a model assuming a constant density value with one with an islet effect.

Subsequently, we repeated this analysis for each island by stratifying the data per habitat type (in MO), or a combination of them (in CA and GU), and occasion. Finally, we considered together those habitats encompassed by the trap array to facilitate comparisons with estimates derived from capture–recapture data (see below). Data truncation was applied when probability of detection was less than 0.15; otherwise, extra parameters were needed to fit the long tail function. The cut-point for right truncation is arbitrary but it is advised to cut at <0.15 of the detection probability or 5% of the observation (Buckland *et al.* 2001; Thomas *et al.* 2010). Transects in Habitat C and in Habitat D at GU were discarded when stratified for occasion. For each island, observations were analysed with DISTANCE 6.0 Release 2 (Thomas *et al.* 2010). Following Buckland *et al.* (2001), graphical fit was used to group observations into distance classes and the best model for each island was selected on the basis of the AIC value.

Capture–mark–recapture

Capture–mark–recapture data were collected in each island with an array of pit-fall traps (n = 26 in CA, n = 25 in GU, and n=31 in MO). Traps were unevenly spaced and positioned ~4m apart along shrub edges and within the vegetation. In each islet, the array encompassed two types of habitat. In CA, it included Habitats B and D (Habitat A and Habitat C were absent). In GU, it included Habitats A–C, and in MO Habitats A and C (Habitat D was absent). Individuals were recognised by photo-identification, according to the method proposed in Sacchi *et al.* (2010), with the aid of a customised computer procedure (O. Moya, P.-L. Mansilla, S. Madrazo, J.-M. Igual, A. Rotger, G. Tavecchia, unpubl. data). Observations were coded as capture–recapture histories, a series of '1' and '0' coding at each occasion for presence and absence, respectively. We assessed whether all individuals had an equal probability of recapture in CA and GU, by using the Cormack's test designed for three-occasion

studies (Cormack 1966 cited in Krebs and Houston 1989). In MO, where data were collected over five occasions, the homogeneity across individuals in the probability of recapture was also assessed through contingency tables and directional *Z*-tests, using software U_CARE (Choquet *et al*. 2005; appendix I in Tavecchia *et al*. 2008). After examining for trap homogeneity, we obtained a direct estimate of lizard density in each islet by using spatially explicit models with package 'SECR' (Efford 2012) in software R (R Core Team 2012). SECR models use the location of each encounter to fit a spatial model of the detection process, which is assumed to follow a distribution with a given mean and a variance, referred to as g_0 and *s*, hereafter (Efford 2004). The parameters g_0 and σ are estimated by maximum likelihood. We assumed σ to be constant and we fit models with constant and time-dependent g_0, namely 'g_0(.)' and 'g_0(t)', respectively. Detector type was set to 'multiple' because several animals might be captured by the same trap during the same session. We considered the model with the lowest AIC as the best compromise between model residual deviance and model complexity (Burnham and Anderson 2001). We first selected the type of function for g_0 by contrasting a model assuming a negative exponential function with one assuming a half-normal function. We then assumed a temporal variation in g_0 and retained the model with the lowest AIC value. After modelling density in each islet separately, we merged the data into a single analysis and compared a model assuming an islet effect with one assuming the same density across islets as a general test for an islet effect. Finally, we obtained a third measure of population density without considering a spatial component. For this, we first estimated the population size, *N*, using the Schnabel's index (Seber 2002). We then divided this estimate by the minimum convex polygon (MCP) encompassing the traps, with the addition of an external strip 0.5 nTD metres wide, where nTD is the average nearest-neighbour distance in metres between traps calculated by the SECR package (Efford 2012). Seber (2002, p. 51) used as a strip width, *W*, the mean diameter of the home range of lizards during the

trapping period. However, we wanted an estimate of a strip width independent from the spatial information on individuals. In this case, lizards do not need to be individually marked. This final density measure was referred to as 'CMR'.

Results

Line transect

We had a total of 2050 contacts with an average of 35 lizards per transect per day on each island (Table 1). When all data were merged, the AIC of a model assuming that density changes across island was 23.44 points lower than that of a model assuming a constant value, suggesting an overall difference in density across the three populations. When the data for each islet were analysed separately, we found that the half-normal function for detection probability was the more appropriate to describe the detection probability, except for MO, in which a cosine expansion was necessary (Table 2). Density estimates across islets changed from 1306 to 2093 individuals ha^{-1}; this range was likely to be the consequence of the difference in habitat composition rather than islet area *per se* (Fig. 1). Indeed, within an islet, estimates had a similar range of values when stratified by habitat type (Table 2). Also, densities in Habitat C in GU and MO were lower than in other habitats, but nearly two times higher in MO than in GU. Density tended to be higher in habitat or combination of habitats with low vegetation (Habitat B; Fig. 2). However, we were not able to statistically test for a difference among habitats because habitat-type combinations differed across islets.

Islet	Habitat	TL (m)	$\hat{D}$ (ind. ha^{-1})	95% CL	F	CV (%)
Es Caragol (CA)	**B+D**	**147**	**1799**	**1505–2151**	**HN**	**8.9**
Na Guardia (GU)	A+B+C+D	617	1306	1139–1498	HN	7
	A+B+C	**439**	**1784**	**1551–2051**	**HN**	**7.1**
	A+B	301	2622	2256–3048	HN	7.7
	C	138	649	443–949	HN	19.1
	D	178	–	–	–	–
Na Moltona (MO)	A+B+C+D	1227	2003	1806–2222	HNC	4.9
	A+C	**942**	**2093**	**1732–2529**	**HNC**	**7.6**
	A	661	1884	1499–2368	HNC	9.1
	B	285	3351	2528–4442	HNC	11
	C	281	1463	1080–1981	HNC	13.4

Table 2. Density estimates ($\hat{D}$) per habitat type in the three islets by line-transect method, using distance-sampling technique Surveys were conducted during 3 days in CA and GU and 5 days in MO. A= high vegetation, B= low vegetation, C= shore and scattered plants, D= rocks, TL = total daily transect length, CL = confidence limits, F = distribution function to describe the distribution of detection probability (HN = half-normal, HNC= half-normal and cosine), and CV= coefficient of variation. Models selected for comparison with capture–recapture spatially explicit models (SECR) are in bold

On top of these differences, estimates varied among occasions. At GU islet, for example, density was estimated to be 1534 individuals ha^{-1} at the second occasion and 2651 individuals ha^{-1} the day after. Similarly, at MO, densities ranged from 1990 individuals ha^{-1} to 3708 individuals ha^{-1} over the 5 days of the survey (results not shown). This variability, caused in part by the lizard response to weather changes, reflects also a higher sampling error of the detection process in vegetated habitats (Fig. 2). In MO, where three habitat types were surveyed simultaneously and during five occasions, the coefficient of variation (CV) per habitat was 0.38, 0.19 and 0.16 in high vegetation, low vegetation and coastal habitat, respectively (Fig. 2). Indeed, in vegetated habitats, the detection function had a more difficult adjustment because of a lower than expected number of observations at a short distance (Fig. 3). LT estimates obtained by analysing sequentially the 5-day data at MO were variable, with no particular relation with the number of occasions (CV: 0.11; Fig. 4). Note

that the confidence intervals of the daily estimates are independent and relate to the amount and sparseness of the data collected that day.

Capture–recapture

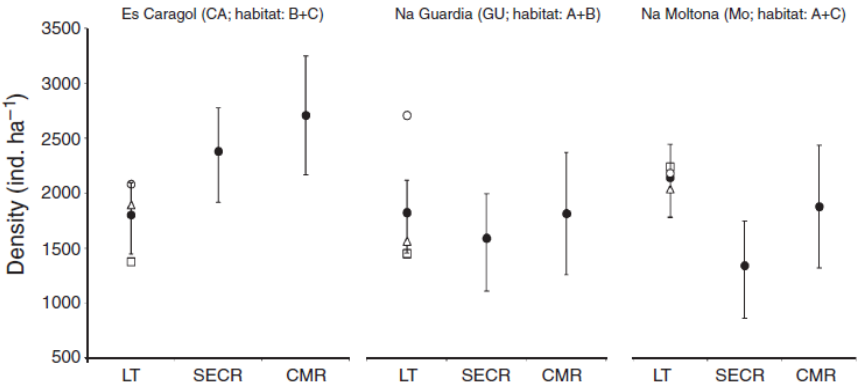

Fig. 1. Autumn population density of Balearic lizard estimated by line-transect (LT) and capture–recapture spatially explicit (SECR) models and Schnabel index (CMR) in three islets, with average value estimated over three occasions (•), at Occasion 1 (□), at Occasion 2 (Δ) and at Occasion 3 (○). Islet surface is increasing from left to right. Vertical bars indicate 95% confidence intervals for the average estimates. Habitats: A = high vegetation, B = low vegetation, C = shore and scattered plants, and D = rocks only.

The goodness of fit test supported the assumption of equal recapture probability across individuals in all 3-day datasets (CA: Z = 0.162, *P* = 0.106; GU: Z = 0.654, *P* = 0.32; MO: Z = 1.309, *P* = 0.169). Similarly, in all three islets, a negative exponential function for g_0 was preferred to the half-normal distribution (Table 3), indicating that recapture probability abates rapidly with the distance from the trap. The model, g_0 (t), assuming a different recapture probability in each occasion was preferred in all populations, reflecting a change in the recapture process. The goodness of fit of the 5-day survey at MO supported the hypothesis of a homogeneous capture probability across individuals (c22 = 1.854, *P* = 0.396) and the absence of a trap response (Z = 0.53, *P* = 0.596). Estimates

from the retained models indicated that the highest density of lizards was in CA (2381 individuals ha^{-1}; 95% CL: 1989–2851), followed by GU (1560 individuals ha^{-1}; 95% CL: 1141–2132) and MO (1316 individuals ha^{-1}; 95%CL: 963–1798). As the structure of g_0 was the same in all islets, we merged the datasets and contrasted a model assuming a different density across islets with one including a constant value. The AICc of the model assuming an islet-dependent density was 4.451 points lower, suggesting an overall statistically significant difference in density across islets. CMR estimates, without spatial information, were systematically higher but comparable with those obtained by SECR models (Figs 2, 4). However, point estimates at MO using the CMR method decreased with the number of occasions (from 2591 individuals ha^{-1} to 1637 individuals ha^{-1} when two or five occasions were considered, respectively; CV: 0.22). The highest drop, however, was between two and three occasions (Fig. 4). Interestingly, this was not the case for the SECR-derived estimates (CV: 0.02). As expected, the standard error of the estimates decreased with the number of occasions considered for SECR as well as conventional CMR methods (Fig. 4). Note that CMR results of Fig. 4 islet would be 22% higher when *W* is used instead of nTD (Table 1). This difference increases to nearly 30% in the other two islands and can be calculated as $1 - A/A$ (nTD).

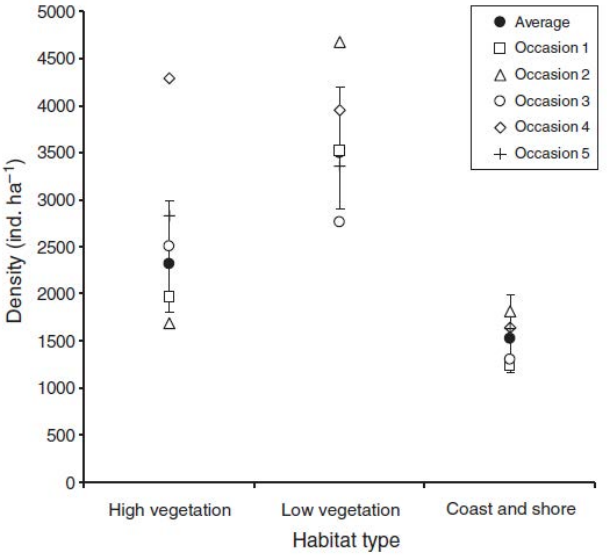

Fig. 2. Autumn density (individuals ha–1) at Na Moltona (MO) islet according to habitat type and occasions, estimated by line transect. The coefficient of variation per habitat was 0.38, 0.19 and 0.16 in high vegetation, low vegetation and in coastal-shore habitat, respectively.

Discussion

We empirically compared the density estimates of three island populations of lizards derived by two common, but seldom contrasted, sampling designs, namely, LT surveys and capture–recapture sampling. In addition, capture–recapture data were analysed using models including the capture locations (SECR) or those without the locations (CMR), to assess the influence of accounting for animal home range in parameter estimates. The performance of a given method should be assessed using simulated data in which the real parameters are known (Rodda and Campbell 2002; Tenan *et al*. 2013). However, simulated data do not typically include the many constraints and variance components that one might experience in empirical data and, despite their limitation, a 'soft' validation (*sensu* Rodda and Campbell 2002; Rodda 2012) of density

estimates can be informative. A first limitation of our study was that real densities were unknown, preventing the assessment of the accuracy for a given method. Nevertheless, important empirical indications on method performance arose from the comparisons across the approaches used (see below). Results suggested that differences across islands found by LT and SECR models were mediated by the different proportion of vegetated habitat in each population (Tables 1, 2).

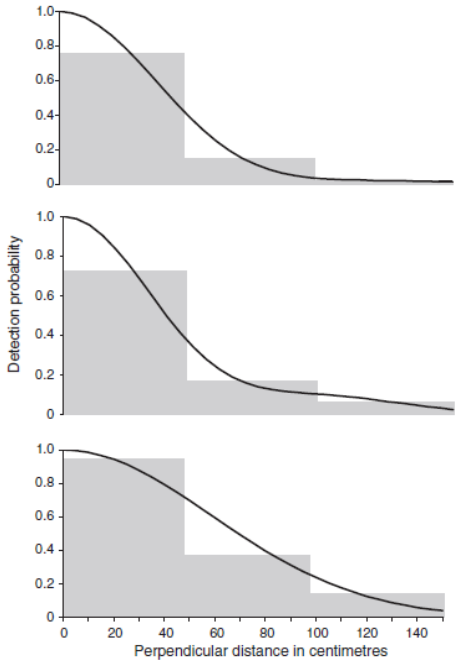

Fig. 3. Detection functions of lizards observed from line transects at Na Moltona islet according to habitat from 5-day survey. Columns = observed, line = expected from the selected model; see text for details. Top = high vegetation (half-normal cosine detection function), centre = low vegetation (half-normal cosine detection function), and bottom = coast and shore (half-normal).

In particular, results from LT surveys indicated that greater density of lizards was found in low vegetation, possibly owing to food availability, habitat conditions and thermoregulatory behaviour. Also, estimates in high vegetation had a larger CV, suggesting that the use of this habitat changed during the 5 days of sampling. We used a qualitative characterisation of habitat type based on macro-similarities such as plant

species and shrub height. It is possible that a more quantitative measure based on, for example, habitat fragmentation or plant cover would provide a better description of density variability. LT methods assumed that all animals are detected previous to their movement, that all individuals on the transect line are detected with certainty and that detection abates with the distance from the line. We have found evidence of possible departures from model assumptions, depending on the habitat considered. Indeed, LT models predicted more lizards close to the transect in vegetated habitats than was actually observed and the half-normal distribution function for detection probability has to be extended to account for a larger number of lizards observed distant from the line than what was expected (Fig. 3). This is probably due to the fact that lizards perceived the approaching observer as a danger and were detected only after the movement occurred, in contrast with what is assumed by the model in that animals are recorded before they move either toward or away from the observer. This phenomenon seems less pronounced in open habitats, where lizards are likely to be seen sooner.

A possible departure from model assumptions might also explain why LT densities were higher than CMR and SECR estimates in islets with high vegetation, but not in CA where vegetation is mainly low or absent (Fig. 1). Note that LT densities might be biased upward as well as downward, depending on animal and observer speed, on the graphical adjustment and the cut-off point of the detection curve (Buckland *et al*. 2001). Anderson *et al*. (2001) and Smolensky and Fitzgerald (2010) found that the violation of complete detection of individuals on the transect line led to underestimates of population density. These studies concluded that densities are generally underestimated because of 2012) of density estimates can be informative. A first limitation of our study was that real densities were unknown, preventing the assessment of the accuracy for a given method. Nevertheless, important empirical indications on method performance arose from the comparisons ac oss the approaches used

(see below). Results suggested that differences across islands found by LT and SECR models were mediated by the different proportion of vegetated habitat in each population (Tables 1, 2). In particular, results from LT surveys indicated that greater density of lizards was found in low vegetation, possibly owing to food availability, habitat conditions and thermoregulatory behaviour. Also, estimates in high vegetation had a larger CV, suggesting that the use of this habitat changed during the 5 days of sampling.

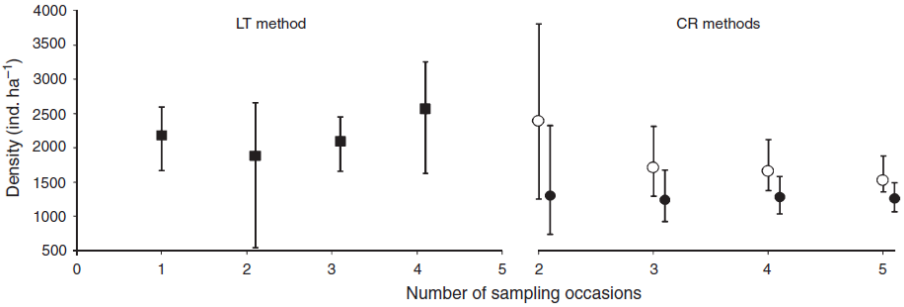

Fig. 4. Density estimates at Na Moltona islet according to the number of sampling occasions obtained by different methods. Left = LT method, right = CR methods. SECR models are indicated by solid symbols, and CMR models by open symbols; see text for details.

We used a qualitative characterisation of habitat type based on macro-similarities such as plant species and shrub height. It is possible that a more quantitative measure based on, for example, habitat fragmentation or plant cover would provide a better description of density variability. LT methods assumed that all individuals on the transect line are detected with certainty and that detection abates with the distance from the line. We have found evidence of possible departures from model assumptions, depending on the habitat considered. Indeed, LT models predicted more lizards close to the transect in vegetated habitats than was actually observed and the half-normal distribution function for detection

probability has to be extended to account for a larger number of lizards observed distant from the line than what was expected (Fig. 3). This is probably due to the fact that lizards perceived the approaching observer as a danger and were detected only after the movement occurred, in contrast with what is assumed by the model in that animals are recorded before they move either toward or away from the observer. This phenomenon seems less pronounced in open habitats, where lizards are likely to be seen sooner. A possible departure from model assumptions might also explain why LT densities were higher than CMR and SECR estimates in islets with high vegetation, but not in CA where vegetation is mainly low or absent (Fig. 1). Note that LT densities might be biased upward as well as downward, depending on animal and observer speed, on the graphical adjustment and the cut-off point of the detection curve (Buckland *et al.* 2001). Anderson *et al.* (2001) and Smolensky and Fitzgerald (2010) found that the violation of complete detection of individuals on the transect line led to underestimates of population density. These studies concluded that densities are generally underestimated because of inactive lizards. In our case, because the real density was unknown, we could not test this hypothesis and inactive lizards are likely to be absent from both LT and CMR datasets, allowing a relative comparison of the methods. CMR density estimates are based on recapture processes estimated from the encounter histories of marked animals. Recently developed SECR models (Efford 2004, 2010) incorporate a model for animal movements built on capture locations. These models gave estimates that were always lower than those obtained without the spatial information on captures (CMR; Fig. 1). A similar result was found by Noss *et al.* (2012) when comparing estimates from capture–recapture data of mammals with and without spatial information (see also Efford *et al.* 2005). Krebs *et al.* (2011) also found that at high density SECR estimates were systematically smaller, whereas the opposite was true at low density. The lower SECR than CMR estimates were probably a consequence of boundary-strip measures being expected to be underestimates of the

effective trapping area (Efford 2009; Krebs *et al.* 2011). CMR estimates are sensitive to the way the boundary strip around the array area is calculated. In our case, for example, using the average maximum distance between detections pooled over individuals (*W*), instead of the nearest-neighbour distance between traps (nTD), would have led to an increase of 20–30% in the population density (Table 1). Finally, MacLulich (1951, in Seber 2002, p. 51) suggested a method for the simultaneous estimation of population density and animal home range, providing the trap arrays are large enough to catch most of the animals whose ranges overlap the trapping area. Boundary strip is then assumed to be half the mean diameter of the home range (Seber 2002, p. 51).

Model and notation	Distribution	AIC	*np*	MLE-D
Es Caragol (CA; habitat type in trap array: B + D)				
1 g0(.)	Half-normal	966.45	3	2213 (1853–2644)
2 g0(.)	Neg. Exp.	915.65	3	2398 (2002–2873)
3 g0(t)	**Neg. Exp.**	**913.5**	**5**	**2381 (1989–2851)**
Na Guardia (GU; habitat type in trap array: A + B + C)				
1 g0(.)	Half-normal	637.62	3	1384 (1028–1863)
2 g0(.)	Neg. Exp.	617.83	3	1603 (1171–2196)
3 g0(t)	**Neg. Exp.**	**606.51**	**5**	**1560 (1141–2132)**
Na Moltona (MO; habitat type in trap array: A + C)				
1 g0(.)	Half-normal	793.731	3	1082 (788–1485)
2 g0(.)	Neg. Exp.	773.556	3	1339 (979–1830)
3 g0(t)	**Neg. Exp.**	**766.623**	**5**	**1316 (963–1798)**

Table 3. Modelling recapture function with capture–recapture spatially explicit (SECR) models at three islets For a given islet, the trap grid encompassed two types of habitat, except on Na Guardia that had three habitat types. A= high vegetation, B = low vegetation, C = shore and scattered plants, D = rocks (see text), . = constant, t = time, distribution = function to describe the distribution of detection probability, half-normal or negative exponential (Neg. Exp.), AIC = Akaike's information criterion, *np* = number of parameters, and MLE-D = maximum-likelihood density estimates and 95% confidence interval (in parentheses). Models retained are in bold.

In a simulated study, Rees *et al.* (2011) found that CMR estimates depend on the number of occasions. Our results study, the real density values

were unknown; however, the most important change was between two and three occasions. Interestingly, SECR estimates, which consider spatial information of recapture, did not vary with the number of occasions and appeared to have a higher precision (Fig. 4). In agreement with Noss *et al*. (2012), we advise to include, when possible, the spatial information to avoid the potential biases resulting from the way the sampled area is measured. With the exception of the smallest islet, CMR estimates are generally lower than those provided by LT. It is possible that some animals, e.g. small animals, do not visit the traps and CMR methods result in a partial sampling of the population. At the moment, we are not able to verify this hypothesis; however, future research might focus in comparing estimates with removal sampling or in manipulating trap density, to further explore this point.

Buckley and Jetz (2007) reported that lizards on islands occur on average at a density of 1920 (_574) individuals ha^{-1}, with these values being over an order of magnitude higher than those on mainland. Pérez-Mellado *et al*. (2008) reported a great variation of LT densities of the Balearic lizard in 43 islets of the Balearic archipelago. These authors found that estimates ranged from 35 to 8000 individuals ha^{-1} (average density: 1500 lizards ha^{-1}, median density 700 individuals ha^{-1}), with no apparent relationship with islet characteristics. We found between 1500 and 2500 individuals ha^{-1}, which is within the same range of the estimates as previously reported (Pérez-Mellado *et al*. 2008) and in agreement with the average value found by Buckley and Jetz (2007).

Implications for sampling design

LT and capture–recapture methods have both pitfalls and payoffs that mostly depend on the realism of the assumptions made (for assumptions, see Introduction). LT methods provide a simple and economic way to estimate wild-population density. The fundamental implication for survey design is that density estimate can be obtained with only one session and

animals do not need to be individually marked nor physically captured. However, we have shown that animal mobility and habitat structure might influence the accuracy of the detection processes and increase the arbitrary aspect of model adjustment (Figs 3, 4). Hence, it is recommended to stratify data per habitat to account for different detection functions. On top of a spatial variability, LT estimates appeared variable over time when compared with those from other methods. CMR methods need more sampling effort than does LT, but have the advantage of collecting individual-based information, to investigate, for example, movement patterns. Overall, CMR methods appeared to better satisfy model assumptions when sampling a small camouflaged, elusive animal in medium to high vegetation. LT estimates from the 5-day survey showed variations within habitat (Fig. 3) and among occasions (Fig. 4), but with no particular association with the number of sampling occasions. Density estimates with conventional CMR models were affected by the number of occasions (see also Rees *et al*. 2011), whereas those derived by spatially explicit models (SECR; Efford 2004) were not.

Acknowledgements

We are grateful to Camilla Aviaaja Enoksen for her help during the field campaign and to Murray Efford for his help in fitting SECR models. We also thank two anonymous referees for the constructive criticism to the first draft of this manuscript. We thank IB-port, Excursion Boat and Lucia Bonnet for the logistic support. JRdl was supported by a 'J. Benedí' fellowship (IMEDEA – CSIC/UIB). The project was founded by the Spanish Minister for Science and Innovation (BFU2009-09359). Permission to handle the animals was agreed by the Conselleria d Agricultura, Medi Ambient i Territori of the Government of Balearic Islands, n.5906-06/2012. Funds were partially provided by the Regional Government of Balearic Islands and FEDER funding.

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APPENDIX

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http://dx.doi.org/10.1071/WR13127

Estimating lizard population density: an empirical comparison between line-transect and capture–recapture methods

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Abstract

Context. In most natural populations, exhaustive counts are not possible and estimates need to be derived from partial sampling by using analytical methods that account for biological processes, sampling errors and detection probability. The methods available have contrasting pitfalls and payoffs in relation to the assumptions made but are seldom contrasted on the same population.

Aims. We compared density estimates derived by different sampling methods. Despite the real density being unknown, the 'soft' validation of density estimates might help to better understand the possible pitfalls and payoffs of each method. This was done in three closed populations and with three different habitat typologies to disentangle the effects of different capture-detection processes to those introduced by the method itself.

Methods. We considered the problem of estimating population density of the endemic Balearic lizard, *Podarcis lilfordi*, in three island populations. We compared estimates derived by distance sampling (LT) in three types of habitat with those calculated from a simultaneous 3-day capture–mark–recapture study. Capture histories of marked individuals were used to estimate density using spatially explicit capture–recapture models (SECR) and a capture–mark–recapture model without spatial data (CMR). Moreover, we empirically assessed the influence of survey duration by extending the survey in the largest island to five occasions. The real population density was unknown and absolute accuracy of each method cannot be assessed; nevertheless, relative estimates might be informative.

Key results. LT estimates had the greatest coefficient of variation in vegetated habitats, corresponding to possible departures from model assumptions. SECR estimates differed among islands and were from 12% to 37% lower than those derived by LT but only in the largest islands with high and dense vegetation. CMR estimates depended on the number of occasions whereas SECR did not and showed lower variance. LT and SECR estimates showed differences across islets.

Conclusions. Line-transect and capture–recapture methods gave comparable results but the interaction between recapture processes and habitat types should be considered when inferring density to the whole area. We found density estimates between 1500 and 2500 individuals ha^{-1}, being a higher value than those found for lizards in continental regions.

Implications. Pitfalls and payoffs of each method are discussed to optimise experimental design in estimating population density.

Additional keywords: detection, distance sampling, habitat, island, SECR.

Received 19 February 2013, accepted 7 November 2013, published online 18 December 2013

Introduction

The distribution of individuals over space and time is a central theme in ecological theory. Yet, how to obtain a robust measure of population abundance (N), or density (D), is still an open field of research (Buckland *et al.* 2001; Seber 2002; Williams *et al.* 2002; Tavecchia *et al.* 2009). The problem has its root in the scientific method itself. When exhaustive counts are not possible, estimates have to be derived from partial sampling by using analytical methods that account for biological processes, sampling errors and detection probability. A first step to reduce problem complexity is to conduct surveys over a short period of time so that the population can be assumed to be closed to emigration, immigration, birth and death and only detection probability has to be considered (Otis *et al.* 1978; Seber 2002; Williams *et al.* 2002). An estimate of the population density, $\hat{D}$, defined as the number of individuals per unit area is generally preferred over an estimate of population size, $\hat{N}$, because it allows comparisons across species and over space and time. The two estimates are related by the area surveyed, A, so that $\hat{D} = \hat{N}/A$. As a consequence, population density models have typically two components, one to account for the detection process and a second to estimate A. The two sets of methods

commonly used for the estimate of population density are line-transect (LT) and capture–mark–recapture methods (CMR). In LT, observers count the number of animals while moving along a transect line and measure, or give an estimate of, the distance from transect line at which each individual has been seen (Buckland *et al.* 2001). In CMR studies, animals are captured, marked and recaptured (or resighted) and an estimate of population abundance can be derived from individual capture histories (Schwarz and Anderson 2001; Seber 2002; Williams *et al.* 2002). Population density can be then derived using an estimate of A, which is typically based on the relative positions of detectors (Sutherland 2006) or on animal movements, such as average home-range size (Seber 2002) or mean maximum distance moved (Wilson and Anderson 1985). These add-on methods are only partially satisfactory. Spatially explicit capture–recapture models (SECR; Efford 2004) incorporate an estimate of A with an underlying model of animal home-range based on the movement of marked animals between detectors (traps). This solution should be preferred to the conventional CMR approach because in SECR models, the estimations of $\hat{D}$ and A occur simultaneously and density estimates or realised population size are less biased (see Borchers and Efford 2008; Noss *et al*. 2012; Efford and Fewster 2013 for more detailed on advantages of SECR).

At a first look, LT and capture–recapture methods differ only in the way recapture processes are treated. However, they have different pitfalls and payoffs in relation to the assumptions made and the system under study. Detection processes in LT are assumed to have the following three characteristics: (1) the detection probability decreases with the distance from the transect line, (2) individuals on the transect line are detected with certainty, and (3) individuals are detected at their initial location and their distance from the transect line is exact (Buckland *et al.* 2001). Capture–recapture methods assume that recapture events are independent, that marks are not lost, that individuals are homogeneous in their capture probability and that previous captures do not influence the following encounter history. Non-spatial explicit model further assumes geographic closure, hence, the need to estimate the sampled area. SECR models do not assume geographic closure, but assume that animal home-range is approximately circular and have a fix central location (Efford 2004). In some systems, LT provides a quick and not expensive way to obtain robust estimates of population density or abundance (Aars *et al.* 2009). In other cases, CMR and SECR methods might be more suitable because animals are difficult to detect or LT model assumptions are not met (Hendriks *et al.* 2012).

We considered the problem of estimating population density of the Balearic lizard, *Podarcis lilfordi* (Squamata, Lacertidae; Günther, 1874), in three island populations. The isolated character of island populations, the reduced species diversity and the relatively small number of interactions across species alter the balance of environmental and ecological control on population dynamics (MacArthur and Wilson 1967; Wright 1980). Because of this ecological setting and the low energetic costs, lizards on islands have potential to reach exceptionally high densities following predator release, diet change and competitor release, a phenomenon referred to as 'density compensation' (MacArthur *et al.* 1972; Olesen and Valido 2003). Buckley and Jetz (2007) found that lizards on islands occur at densities about an order of magnitude higher than those recorded on mainland. Within the Mediterranean basin, the Balearic Islands (Spain) are one of the regions in which endemic lizards attain very dense populations (Salvador 1986; Pérez-Mellado 1989, 1998); however, the exact density value has seldom been estimated (but see Pérez-Mellado *et al*. 2008). Reptile populations are notoriously difficult to measure because of the small body size of the animals, secretive behaviour, habitat preferences and fast unpredictable activity (Turner 1977). Despite these constraints, there is a long tradition of using transect sampling to estimate population densities of herpetofauna (Iverson 1978; Cassey and Ussher 1999; Germano *et al.* 2003; Reisinger *et al.* 2006; Pérez-Mellado *et al.* 2008). In some species of reptiles, however, individuals are difficult to detect and tend to be seen during movements, rather than before as assumed by LT models. The violation of model assumptions leads to biased estimates of density, to the point that some authors discourage the use of visual counts in estimating lizard density (Smolensky and Fitzgerald 2010). Other authors used capture–recapture methods to estimate population density and abundance (Ballinger and Congdon 1981; Kwiatkowski and Sullivan 2002); however, few have compared the two methodologies (Kacoliris *et al.* 2009; see also Funk *et al.* 2003; Smolensky and Fitzgerald 2010). Kacoliris *et al.* (2009) suggested that capture–recapture data might lead to less biased estimates; however, this might depend on animal behaviour and habitat characteristics (V. Pérez-Mellado, pers. comm.).

We compared lizard density estimates derived by (1) distance sampling (Buckland *et al.* 2001), (2) spatially explicit capture–recapture models (Efford and Fewster 2013) and (3) capture–recapture without spatial data (Seber 2002). The real density of lizards was unknown, but the 'soft' validation of density estimates (*senus* Rodda and Campbell 2002) might help better understand the possible pitfalls and payoffs of each method. We did this in three closed populations and with three different habitat typologies to disentangle the effects of different capture–detection processes to those introduced by the method itself.

Materials and methods

Species and study area

The Balearic lizard is an endemic lizard of the Balearic archipelago (Spain). It is considered endangered by the IUCN criteria (Pérez-Mellado and Martínez-Solano 2009), and vulnerable at the regional level (Viada 2006). Its distribution is currently confined to the islets of Mallorca and Menorca and to the Cabrera archipelago. We estimated lizard density in the following three islets in the south coast of Mallorca: Es Caragol (0.29 ha, named 'CA' hereafter), Na Guardia (1.98 ha, named 'GU' hereafter) and Na Moltona (5.09 ha, named 'MO' hereafter). Vegetation richness and structure changed with islet size, with 'CA' having a low and less diverse vegetation and 'MO' with a high and more diverse one. We recognised four main habitat categories and characterised each island on the basis of their occurrence (Table 1). The first habitat type, named 'A', was characterised by a vegetation higher than 50 cm, including mainly shrubs of genera *Pistacia* and *Phillyrea*, the second one, 'B', was

Table 1. Islet area, proportions of habitat type in the islet, number of transects and characteristic of trap-arrays

A = high vegetation, B = low vegetation, C = shore and scattered plants, D = rocks, nTD = nearest-neighbour distance between traps, W = average maximum distance between detections pooled over individuals, A = array area estimated by the minimum convex polygon (MCP), and A(nTD) = area of the array obtained by adding a boundary strip of constant width of 0.5 nTD. Note that Habitats A and B in GU are combined

Parameter	Es Caragol (CA)	Na Guardia (GU)	Na Moltona (MO)
Total area of the islet (ha)	0.29	1.98	5.06
High vegetation (Type A)	–	0.43	0.53
Low vegetation (Type B)	0.69		0.18
Shore (Type C)	–	0.11	0.29
Rocks (Type D)	0.31	0.46	–
Number of transects per day	3	5	7
Number of day	3	3	5
Number of traps	26	25	31
Trap array perimeter (m)	118	157	181
nTD (m)	5.9	8.1	6.3
W (m)	5.6	5.7	8.0
A(nTD) (ha)	0.125	0.233	0.270
A (ha)	0.088	0.164	0.210
Total number of lizards observed or captured			
Line transect	164	379	1507 (881[^])
Capture in grid	232	188	397 (254[^])
Recapture in grid	80	35	94 (36[^])

[^]Three days only.

characterised by vegetation less than 50 cm (i.e. *Crithmum maritimum*), the third, 'C', comprised sand, rocks and few plants of halophytic species, such as *Salicornia ramosissima*, and the fourth, named 'D', comprised exclusively rocks and pools of salted water (Table 1). These last two categories are suboptimal habitats for lizards and marked the region between the vegetated area and the sea. Nevertheless, we do not know the importance of these habitats that lizards use for shelter and in search of food.

Line transect

In October 2012, each islet was visited during three consecutive days, with the exception of MO that was visited two additional days to evaluate the effect of a longer sampling period on density estimates. According with island surface, we considered three line transects per day at CA, five line transects per day at GU and seven line transects per day at MO, making a total of 59 line transects (Table 1). In the largest islet (MO), we were able to survey each habitat separately but this was not possible in the other two islets where transect lines would have been too short (except Habitat C in GU). Following Buckland *et al*. (2001), an observer travelled along a line of variable and known length and recorded the perpendicular distance of each observed lizard to the transect in 10-cm classes. Transects were performed daily by the same observer. We first ran a global analysis merging the data from all islets and contrasting a model assuming a constant density value with one with an islet effect. Subsequently, we repeated this analysis for each island by stratifying the data per habitat type (in MO), or a combination of them (in CA and GU), and occasion. Finally, we considered together those habitats encompassed by the trap array to facilitate comparisons with estimates derived from capture–recapture data (see below). Data truncation was applied when probability of detection was less than 0.15; otherwise, extra parameters were needed to fit the long tail function. The cut-point for right truncation is arbitrary but it is advised to cut at <0.15 of the detection probability or 5% of the observation (Buckland *et al.* 2001; Thomas *et al.* 2010). Transects in Habitat C and in Habitat D at GU were discarded when stratified for occasion. For each island, observations were analysed with DISTANCE 6.0 Release 2 (Thomas *et al.* 2010). Following Buckland *et al*. (2001), graphical fit was used to group observations into distance classes and the best model for each island was selected on the basis of the AIC value.

Capture–mark–recapture

Capture–mark–recapture data were collected in each island with an array of pit-fall traps (n = 26 in CA, n = 25 in GU, and n = 31 in MO). Traps were unevenly spaced and positioned ~4 m apart along shrub edges and within the vegetation. In each islet, the array encompassed two types of habitat. In CA, it included Habitats B and D (Habitat A and Habitat C were absent). In GU, it included Habitats A–C, and in MO Habitats A and C (Habitat D was absent). Individuals were recognised by photo-identification, according to the method proposed in Sacchi *et al*. (2010), with the aid of a customised computer procedure (O. Moya, P.-L. Mansilla, S. Madrazo, J.-M. Igual, A. Rotger, G. Tavecchia, unpubl. data). Observations were coded as capture–recapture histories, a series of '1' and '0' coding at each occasion for presence and absence, respectively. We assessed whether all individuals had an equal probability of recapture in CA and GU, by using the Cormack's test designed for three-occasion studies (Cormack 1966 cited in Krebs and Houston 1989). In MO, where data were collected over five occasions, the homogeneity across individuals in the probability of recapture was also assessed through contingency tables and directional Z-tests, using software U_CARE (Choquet *et al.* 2005; appendix I in Tavecchia *et al.* 2008). After examining for trap homogeneity, we obtained a direct estimate of lizard density in each islet by using spatially explicit models with package 'SECR' (Efford 2012) in software R (R Core Team

2012). SECR models use the location of each encounter to fit a spatial model of the detection process, which is assumed to follow a distribution with a given mean and a variance, referred to as $g0$ and s, hereafter (Efford 2004). The parameters $g0$ and s are estimated by maximum likelihood. We assumed s to be constant and we fit models with constant and time-dependent $g0$, namely '$g0$(.)' and '$g0$(t)', respectively. Detector type was set to 'multiple' because several animals might be captured by the same trap during the same session. We considered the model with the lowest AIC as the best compromise between model residual deviance and model complexity (Burnham and Anderson 2001). We first selected the type of function for $g0$ by contrasting a model assuming a negative exponential function with one assuming a half-normal function. We then assumed a temporal variation in $g0$ and retained the model with the lowest AIC value. After modelling density in each islet separately, we merged the data into a single analysis and compared a model assuming an islet effect with one assuming the same density across islets as a general test for an islet effect. Finally, we obtained a third measure of population density without considering a spatial component. For this, we first estimated the population size, N, using the Schnabel's index (Seber 2002). We then divided this estimate by the minimum convex polygon (MCP) encompassing the traps, with the addition of an external strip 0.5 nTD metres wide, where nTD is the average nearest-neighbour distance in metres between traps calculated by the SECR package (Efford 2012). Seber (2002, p. 51) used as a strip width, W, the mean diameter of the home range of lizards during the trapping period. However, we wanted an estimate of a strip width independent from the spatial information on individuals. In this case, lizards do not need to be individually marked. This final density measure was referred to as 'CMR'.

Results

Line transect

We had a total of 2050 contacts with an average of 35 lizards per transect per day on each island (Table 1). When all data were merged, the AIC of a model assuming that density changes across island was 23.44 points lower than that of a model assuming a constant value, suggesting an overall difference in density across the three populations. When the data for each islet were analysed separately, we found that the half-normal function for detection probability was the more appropriate to describe the detection probability, except for MO, in which a cosine expansion was necessary (Table 2). Density estimates across islets changed from 1306 to 2093 individuals ha^{-1}; this range was likely to be the consequence of the difference in habitat composition rather than islet area *per se* (Fig. 1). Indeed, within an islet, estimates had a similar range of values when stratified by habitat type (Table 2). Also, densities in Habitat C in GU and MO were lower than in other habitats, but nearly two times higher in MO than in GU. Density tended to be higher in habitat or combination of habitats with low vegetation (Habitat B; Fig. 2). However, we were not able to statistically test for a difference among habitats because habitat-type combinations differed across islets. On top of these differences, estimates varied among occasions. At GU islet, for example, density was estimated to be 1534 individuals ha^{-1} at the second occasion and 2651 individuals ha^{-1} the day after. Similarly, at MO, densities ranged from 1990 individuals ha^{-1} to 3708 individuals ha^{-1} over the 5 days of the survey (results not shown). This variability, caused in part by the lizard response to weather changes, reflects also a higher sampling error of the detection process in vegetated habitats (Fig. 2). In MO, where three habitat types were surveyed simultaneously and during five occasions, the coefficient of variation (CV) per habitat was 0.38, 0.19 and 0.16 in high vegetation, low vegetation and coastal habitat, respectively (Fig. 2). Indeed, in vegetated habitats, the detection function had a more difficult adjustment because of a lower than expected number of observations at a short distance (Fig. 3). LT estimates obtained by analysing sequentially the 5-day data at MO were variable, with no particular relation with the number of occasions (CV: 0.11; Fig. 4). Note that the confidence intervals of the daily estimates are independent and relate to the amount and sparseness of the data collected that day.

Capture–recapture

The goodness of fit test supported the assumption of equal recapture probability across individuals in all 3-day datasets (CA: $Z = 0.162$, $P = 0.106$; GU: $Z = 0.654$, $P = 0.32$; MO:

Table 2. Density estimates ($\hat{D}$) per habitat type in the three islets by line-transect method, using distance-sampling technique
Surveys were conducted during 3 days in CA and GU and 5 days in MO. A = high vegetation, B = low vegetation, C = shore and scattered plants, D = rocks, TL = total daily transect length, CL = confidence limits, F = distribution function to describe the distribution of detection probability (HN = half-normal, HNC = half-normal and cosine), and CV = coefficient of variation. Models selected for comparison with capture–recapture spatially explicit models (SECR) are in bold

Islet	Habitat	TL (m)	$\hat{D}$ (ind. ha^{-1})	95% CL	F	CV (%)
Es Caragol (CA)	**B+D**	**147**	**1799**	**1505–2151**	**HN**	**8.9**
Na Guardia (GU)	A+B+C+D	617	1306	1139–1498	HN	7
	A+B+C	**439**	**1784**	**1551–2051**	**HN**	**7.1**
	A+B	301	2622	2256–3048	HN	7.7
	C	138	649	443–949	HN	19.1
	D	178	–	–	–	–
Na Moltona (MO)	A+B+C+D	1227	2003	1806–2222	HNC	4.9
	A+C	**942**	**2093**	**1732–2529**	**HNC**	**7.6**
	A	661	1884	1499–2368	HNC	9.1
	B	285	3351	2528–4442	HNC	11
	C	281	1463	1080–1981	HNC	13.4

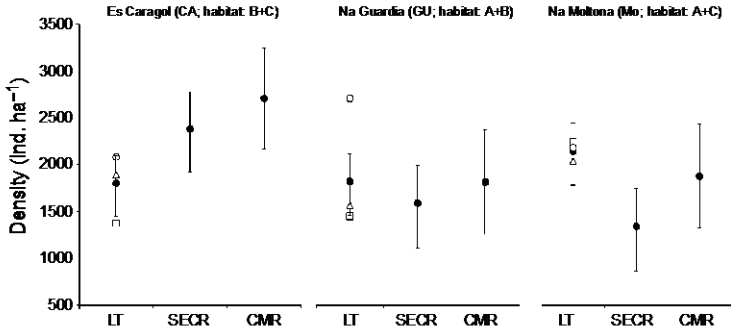

Fig. 1. Autumn population density of Balearic lizard estimated by line-transect (LT) and capture–recapture spatially explicit (SECR) models and Schnabel index (CMR) in three islets, with average value estimated over three occasions (*), at Occasion 1 (&), at Occasion 2 (~) and at Occasion 3 (*). Islet surface is increasing from left to right. Vertical bars indicate 95% confidence intervals for the average estimates. Habitats: A = high vegetation, B = low vegetation, C = shore and scattered plants, and D = rocks only.

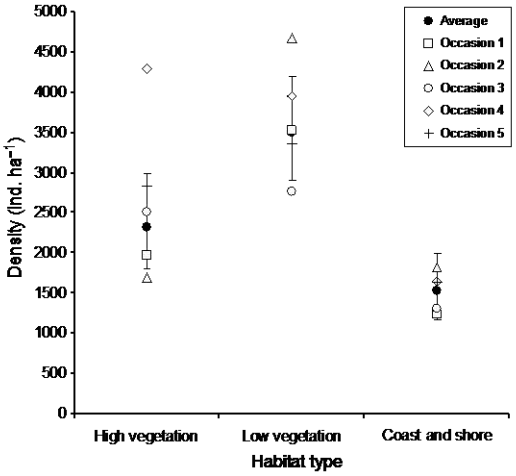

Fig. 2. Autumn density (individuals ha^{-1}) at Na Moltona (MO) islet according to habitat type and occasions, estimated by line transect. The coefficient of variation per habitat was 0.38, 0.19 and 0.16 in high vegetation, low vegetation and in coastal-shore habitat, respectively.

$Z = 1.309$, $P = 0.169$). Similarly, in all three islets, a negative exponential function for $g0$ was preferred to the half-normal distribution (Table 3), indicating that recapture probability abates rapidly with the distance from the trap. The model, $g0(t)$, assuming a different recapture probability in each occasion was preferred in all populations, reflecting a change in the recapture process. The goodness of fit of the 5-day survey at MO supported the hypothesis of a homogeneous capture probability across individuals ($c^2_2 = 1.854$, $P = 0.396$) and the absence of a trap response ($Z = 0.53$, $P = 0.596$). Estimates from the retained models indicated that the highest density of lizards was in CA (2381 individuals ha^{-1}; 95% CL: 1989–2851), followed by GU (1560 individuals ha^{-1}; 95% CL: 1141–2132) and MO (1316 individuals ha^{-1}; 95%CL: 963–1798). As the structure of $g0$ was the same in all islets, we merged the datasets and contrasted a model assuming a different density across islets with one including a constant value. The AICc of the model assuming an islet-dependent density was 4.451 points lower, suggesting an overall statistically significant difference in density across islets. CMR estimates, without spatial information, were systematically higher but comparable with those obtained by SECR models (Figs 2, 4). However, point estimates at MO using the CMR method decreased with the number of occasions (from 2591 individuals ha^{-1} to 1637 individuals ha^{-1} when two or five occasions were considered, respectively; CV: 0.22). The highest drop, however, was between two and three occasions (Fig. 4). Interestingly, this was not the case for the SECR-derived estimates (CV: 0.02). As expected, the standard error of the estimates decreased with the number of occasions considered for SECR as well as conventional CMR methods (Fig. 4). Note that CMR results of Fig. 4 islet would be 22% higher when W is used instead of nTD (Table 1). This difference increases to nearly 30% in the other two islands and can be calculated as $1 - A/A$ (nTD).

Discussion

We empirically compared the density estimates of three island populations of lizards derived by two common, but seldom contrasted, sampling designs, namely, LT surveys and capture–recapture sampling. In addition, capture–recapture data were analysed using models including the capture locations (SECR) or those without the locations (CMR), to assess the influence of accounting for animal home range in parameter estimates. The performance of a given method should be assessed using simulated data in which the real parameters are known (Rodda and Campbell 2002; Tenan *et al.* 2013). However, simulated data do not typically include the many constraints and variance components that one might experience in empirical data and, despite their limitation, a 'soft' validation (*sensu* Rodda and Campbell 2002; Rodda

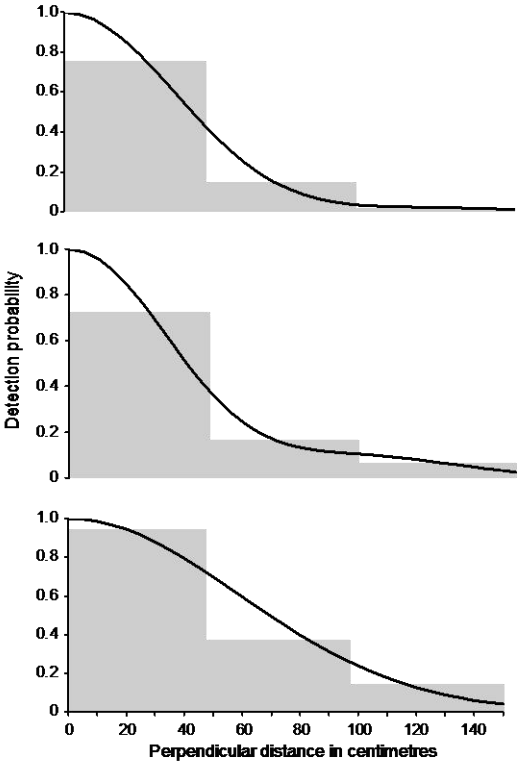

Fig. 3. Detection functions of lizards observed from line transects at Na Moltona islet according to habitat from 5-day survey. Columns = observed, line = expected from the selected model; see text for details. Top = high vegetation (half-normal cosine detection function), centre = low vegetation (half-normal cosine detection function), and bottom = coast and shore (half-normal).

2012) of density estimates can be informative. A first limitation of our study was that real densities were unknown, preventing the assessment of the accuracy for a given method. Nevertheless, important empirical indications on method performance arose from the comparisons across the approaches used (see below). Results suggested that differences across islands found by LT and SECR models were mediated by the different proportion of vegetated habitat in each population (Tables 1, 2). In particular, results from LT surveys indicated that greater density of lizards was found in low vegetation, possibly owing to food availability, habitat conditions and thermoregulatory behaviour. Also, estimates in high vegetation had a larger CV, suggesting that the use of this habitat changed during the 5 days of sampling. We used a qualitative characterisation of habitat type based on macro-similarities such as plant species and shrub height. It is possible that a more quantitative measure based on, for example, habitat fragmentation or plant cover would provide a better description of density variability. LT methods assumed that all animals are detected previous to their movement, that all individuals on the transect line are detected with certainty and that detection abates with the distance from the line. We have found evidence of possible departures from model assumptions, depending on the habitat considered. Indeed, LT models predicted more lizards close to the transect in vegetated habitats than was actually observed and the half-normal distribution function for detection probability has to be extended to account for a larger number of lizards observed distant from the line than what was expected (Fig. 3). This is probably due to the fact that lizards perceived the approaching observer as a danger and were detected only after the movement occurred, in contrast with what is assumed by the model in that animals are recorded before they move either toward or away from the observer. This phenomenon seems less pronounced in open habitats, where lizards are likely to be seen sooner. A possible departure from model assumptions might also explain why LT densities were higher than CMR and SECR estimates in islets with high vegetation, but not in CA where vegetation is mainly low or absent (Fig. 1). Note that LT densities might be biased upward as well as downward, depending on animal and observer speed, on the graphical adjustment and the cut-off point of the detection curve (Buckland *et al.* 2001). Anderson *et al.* (2001) and Smolensky and Fitzgerald (2010) found that the violation of complete detection of individuals on the transect line led to underestimates of population density. These studies concluded that densities are generally underestimated because of

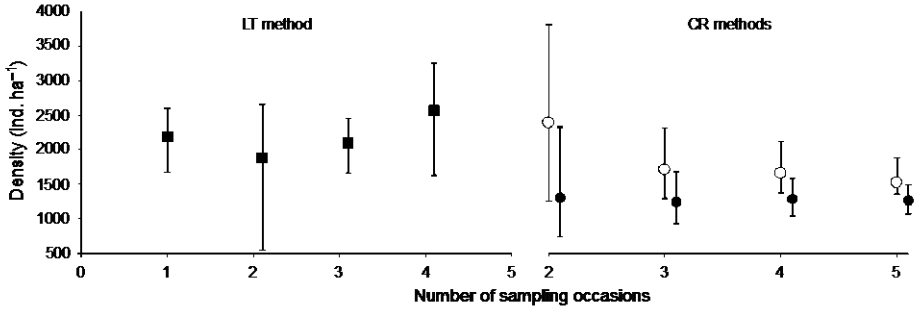

Fig. 4. Density estimates at Na Moltona islet according to the number of sampling occasions obtained by different methods. Left = LT method, right = CR methods. SECR models are indicated by solid symbols, and CMR models by open symbols; see text for details.

Table 3. Modelling recapture function with capture–recapture spatially explicit (SECR) models at three islets

For a given islet, the trap grid encompassed two types of habitat, except on Na Guardia that had three habitat types. A = high vegetation, B = low vegetation, C = shore and scattered plants, D = rocks (see text), . = constant, t = time, distribution = function to describe the distribution of detection probability, half-normal or negative exponential (Neg. Exp.), AIC = Akaike's information criterion, *np* = number of parameters, and MLE-D = maximum-likelihood density estimates and 95% confidence interval (in parentheses). Models retained are in bold

Model and notation	Distribution	AIC	*np*	MLE-D
Es Caragol (CA; habitat type in trap array: B + D)				
1 *g*0(.)	Half-normal	966.45	3	2213 (1853–2644)
2 *g*0(.)	Neg. Exp.	915.65	3	2398 (2002–2873)
3 *g*0(t)	**Neg. Exp.**	**913.5**	**5**	**2381 (1989–2851)**
Na Guardia (GU; habitat type in trap array: A + B + C)				
1 *g*0(.)	Half-normal	637.62	3	1384 (1028–1863)
2 *g*0(.)	Neg. Exp.	617.83	3	1603 (1171–2196)
3 *g*0(t)	**Neg. Exp.**	**606.51**	**5**	**1560 (1141–2132)**
Na Moltona (MO; habitat type in trap array: A + C)				
1 *g*0(.)	Half-normal	793.731	3	1082 (788–1485)
2 *g*0(.)	Neg. Exp.	773.556	3	1339 (979–1830)
3 *g*0(t)	**Neg. Exp.**	**766.623**	**5**	**1316 (963–1798)**

inactive lizards. In our case, because the real density was unknown, we could not test this hypothesis and inactive lizards are likely to be absent from both LT and CMR datasets, allowing a relative comparison of the methods.

CMR density estimates are based on recapture processes estimated from the encounter histories of marked animals. Recently developed SECR models (Efford 2004, 2010) incorporate a model for animal movements built on capture locations. These models gave estimates that were always lower than those obtained without the spatial information on captures (CMR; Fig. 1). A similar result was found by Noss *et al.* (2012) when comparing estimates from capture–recapture data of mammals with and without spatial information (see also Efford *et al.* 2005). Krebs *et al.* (2011) also found that at high-density SECR estimates were systematically smaller, whereas the opposite was true at low density. The lower SECR than CMR estimates were probably a consequence of boundary-strip measures being expected to be underestimates of the effective trapping area (Efford 2009; Krebs *et al.* 2011). CMR estimates are sensitive to the way the boundary strip around the array area is calculated. In our case, for example, using the average maximum distance between detections pooled over individuals (*W*), instead of the nearest-neighbour distance between traps (nTD), would have led to an increase of 20–30% in the population density (Table 1). Finally, MacLulich (1951, in Seber 2002, p. 51) suggested a method for the simultaneous estimation of population density and animal home range, providing the trap arrays are large enough to catch most of the animals whose ranges overlap the trapping area. Boundary strip is then assumed to be half the mean diameter of the home range (Seber 2002, p. 51).

In a simulated study, Rees *et al.* (2011) found that CMR estimates depend on the number of occasions. Our results provided empirical support for these findings, although, in our study, the real density values were unknown; however, the most important change was between two and three occasions. Interestingly, SECR estimates, which consider spatial information of recapture, did not vary with the number of occasions and appeared to have a higher precision (Fig. 4). In agreement with Noss *et al.* (2012), we advise to include, when possible, the spatial information to avoid the potential biases resulting from the way the sampled area is measured. With the exception of the smallest islet, CMR estimates are generally lower than those provided by LT. It is possible that some animals, e.g. small animals, do not visit the traps and CMR methods result in a partial sampling of the population. At the moment, we are not able to verify this hypothesis; however, future research might focus in comparing estimates with removal sampling or in manipulating trap density, to further explore this point.

Buckley and Jetz (2007) reported that lizards on islands occur on average at a density of 1920 (±574) individuals ha^{-1}, with these values being over an order of magnitude higher than those on mainland. Pérez-Mellado *et al.* (2008) reported a great variation of LT densities of the Balearic lizard in 43 islets of the Balearic archipelago. These authors found that estimates ranged from 35 to 8000 individuals ha^{-1} (average density: 1500 lizards ha^{-1}, median density 700 individuals ha^{-1}), with no apparent relationship with islet characteristics. We found between 1500 and 2500 individuals ha^{-1}, which is within the same range of the estimates as previously reported (Pérez-Mellado *et al.* 2008) and in agreement with the average value found by Buckley and Jetz (2007).

Implications for sampling design

LT and capture–recapture methods have both pitfalls and payoffs that mostly depend on the realism of the assumptions made (for assumptions, see Introduction). LT methods provide a simple and economic way to estimate wild-population density. The fundamental implication for survey design is that density estimate can be obtained with only one session and animals do not need to be individually marked nor physically captured. However, we have shown that animal mobility and habitat structure might influence the accuracy of the detection processes and increase the arbitrary aspect of model adjustment (Figs 3, 4). Hence, it is recommended to stratify data per habitat to account for different detection functions. On top of a spatial variability, LT estimates appeared variable over time when compared with those from other methods. CMR methods need more sampling effort than does LT, but have the advantage of collecting individual-based information, to investigate, for example, movement patterns. Overall, CMR methods appeared to better satisfy model assumptions when sampling a small camouflaged, elusive animal in medium to high vegetation. LT estimates from the 5-day survey showed variations within habitat (Fig. 3) and among occasions (Fig. 4), but with no particular association with the number of sampling occasions. Density estimates with conventional CMR models were affected by the number of occasions (see also Rees *et al.* 2011), whereas those derived by spatially explicit models (SECR; Efford 2004) were not.

Acknowledgements

We are grateful to Camilla Aviaaja Enoksen for her help during the field campaign and to Murray Efford for his help in fitting SECR models. We also thank two anonymous referees for the constructive criticism to the first draft of this manuscript. We thank IB-port, Excursion Boat and Lucia Bonnet for the logistic support. JRdI was supported by a 'J. Benedí' fellowship (IMEDEA – CSIC/UIB). The project was founded by the Spanish Minister for Science and Innovation (BFU2009-09359). Permission to handle the animals was agreed by the Conselleria d Agricultura, Medi Ambient i Territori of the Government of Balearic Islands, n.5906-06/2012. Funds were partially provided by the Regional Government of Balearic Islands and FEDER funding.

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Chapter II

The relative role of population density and climatic factors in shaping the body growth rate of the Balearic wall lizard

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Andreu Rotger, José Manuel Igual, Jeffrey Smith and Giacomo Tavecchia (2016). Canadian Journal of Zoology . 94: 207-215.

Photo source: G. Tavecchia.

The relative role of population density and climatic factors in shaping the body growth rate of the Balearic wall lizard (*Podarcis lilfordi*)

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Abstract

The body growth rate in small reptiles is modulated by per-capita food resources and recent evidences suggested that this constraint is the mechanism underlying differences between cohorts. Per-capita food resources depend on population size and climatic factors but their relative role in explaining the variations in growth rate is unclear. We used morphological data collected over six years on more than five hundreds lizards to model the body growth of an insular population of Balearic wall lizard. We used non-linear equations to describe the appropriate length-at-age relationship. For each sex, a seasonal growth was observed and the oscillatory Von Bertalanffy curve was fitted to the data. Three age classes based on this retained curve were recognized, juveniles (0 to 1 year), sub-adults (1 to 2 years) and adults (2+ years). Finally, in each sex-by-age class we investigated the relative role of density, spring rainfall and temperature in explaining the variation of the growth rate. Results showed that the relative role of density and climatic factors varied according to the age considered. While population size has an important negative effect in the growth rate of juveniles, it did not influence sub-adults or adult growth. On the contrary, rainfall had a positive influence for the growth of sub-adults but only a marginal influence for juveniles. Moreover, temperature had a negative effect only in juveniles. Adult growth rate was near zero and constant over time. The different role of density-dependent and climatic factors in explaining age-dependent

growth rate provides an important insight in understanding lizard population dynamics and life-history tactics.

Key words: density-dependent growth, climate, capture-recapture, oscillatory growth, age classes.

Resum

La taxa de creixement del cos en petits rèptils és regulada pels recursos alimentaris per càpita i, segons evidències recents, es suggereix que aquesta limitació és el mecanisme subjacent a les diferències entre les cohorts. Els recursos alimentaris per càpita depenen de la mida de la població i dels factors climàtics però el seu paper relatiu en l'explicació de les variacions en la taxa de creixement no és clara.

Hem utilitzat les dades morfològiques obtingudes durant sis anys per modelar el creixement corporal d'una sargantana insular (*Podarcis lilfordi;* Günter 1874). Utilitzem equacions no lineals per descriure la relació apropiada de la talla per edat. Per a cada sexe, es va observar creixement estacional i la corba oscil·latòria de Von Bertalanffy va ser ajustada a les dades. Tres classes d'edat van ser reconegudes, i es va investigar el paper relatiu de la densitat, la pluja primaveral i la temperatura per explicar la variació de la taxa de creixement en cada classe de sexe per edat. Els resultats van mostrar que el paper relatiu de densitat i factors climàtics variaven en relació amb l'edat considerada. Mentre que la mida poblacional i la temperatura van tenir un efecte negatiu en la taxa de creixement juvenil, les precipitacions van tindre una influència positiva per al creixement de subadults. El creixement adult era prop de zero i constant en el temps. El diferent paper dels factors dependents de la densitat i climàtics en relació a l'explicació de la taxa de creixement dependent de l'edat proporciona una incursió important en la comprensió de la dinàmica de poblacions i les tàctiques d'història de vida de les sargantanes.

Paraules clau: Creixement dependent de la densitat, clima, captura-recaptura, classes d'edat, Illes Balears, sargantana de Lilford, *Podarcis lilfordi*.

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Introduction

In species with continuous growth, the rate at which individuals attain the size of sexual maturity represents an important life-history trait and is involved in multiple evolutionary tradeoffs (Stearns 1992; Caley and Schwarzkopf 2004; Laver et al. 2012). Large variation in growth rate across individuals and populations may result from differences in genetic components (Niewiarowski and Roosenburg 1993), food availability (Dunham 1978), environmental factors, and their interactions (Sinervo and Adolph 1989; Angilletta et al. 2004). However, inter- and intra-population variations in growth suggest a high degree of phenotypic plasticity (Stamps et al. 1998; Badyaev 2002; Sears and Angilletta 2004), and the realized body size at-age relationship is not only the result of evolutionary links, but also of this complex interplay between local resource abundance (Van Valen 1965; Hentschel 1999) and environmental conditions (Schoener and Schoener 1978; Stamps 1993). Despite these two factors being expected to concurrently influence individual growth rate, their role has been usually assessed separately (Le Gaillard et al. 2010; Mugabo et al. 2013). Consequently, the relative importance of population size and climatic factors on body growth rate is often unclear (but see Henle et al. 2004; Zabel et al. 2006). Moreover, the effects of density dependent and independent factors can vary according to individual sex or age (e.g., Massot et al. 1992; Grant and Benton 2000; Coulson et al. 2001). In lizards, for example, the sexes play different roles in reproduction and typically have divergent growth that results in different adult sizes, i.e., sexual size dimorphism (Badyaev 2002). In general terms, female body size and growth rate is shaped by selective pressures on fecundity, whereas male size and growth rate is thought to be driven largely by male– male competition (Taylor and DeNardo 2005). Stage-specific and sex-specific life-history data are therefore often needed to produce realistic dynamics of structured populations regulated by density and climatic fluctuations (Coulson et al. 2001; Zabel et al. 2006).

Here, we used individual-based data to fit the length-at-age curve of the endemic Balearic wall lizard (Lilford's Wall Lizard, *Podarcis lilfordi* (Günther, 1874)) from capture–recapture data of uniquely identified individuals. Once the length-at-age relationship was described, we quantified the relative role of population size and climatic factors affecting the body growth rate of juvenile, subadults, and adult lizards. Overall, insular populations of lizards attain a density that is 10 times higher than the mainland counterparts (Buckley and Jetz 2007). This high density, promoted by the lack of predators (Buckley and Jetz 2007; Pérez-Mellado et al. 2008), is expected to limit individual growth rate because percapita resources are low (Adler and Levins 1994; Grant 1998). Consequently, a first expectation is that individual growth is negatively related to population size (Le Gaillard et al. 2010). Besides the common features of insular populations, the Mediterranean environment (characterized by dry summers and relatively mild winters) imposes different constraints from those found in oceanic climate (Pérez-Mellado and Corti 1993). The high summer temperatures limit resource availability in islands, promoting herbivory, despotic competition, and in the extreme cases, cannibalism (Pérez-Mellado and Traveset 1999; Cooper et al. 2015; Pérez-Cembranos and Pérez-Mellado 2015; Pérez-Mellado et al. 2015). Thus, climatic conditions such as rainfall and temperature are expected to be positively associated with individual growth rate through their influence on habitat quality and lizard physiological processes, respectively (Lorenzon et al. 1999; Jordan and Snell 2002; Marquis et al. 2008). We thus predicted a difference in the growth rate of males and females due to sexual differences in physiology, morphology, and behavior (Le Galliard et al. 2005, 2010). We assessed these predictions using individual data collected over 6 years on individually recognized lizards of known sex.

Table 1. *Growth models for individuals of unknown age*

Model	Equation	AIC Males	AIC Females	References
Logistic	$L_2 = A \cdot L_1/[L_1 + (A - L_1)e^{-K \cdot D}]$	3983.23	2529.34	Schoener and Schoener 1978
von Bertalanffy	$L_2 = A - (A - L_1)e^{-K \cdot D}$	3964.31	2508.54	Fabens 1965
Schnute†	$L_2 = L_1 \cdot e^{-K \cdot D} + (y_2 - y_1 \cdot e^{-K(T_1 - T_2)}) \cdot 1 - e^{-K \cdot D}/1 - e^{-K \cdot (T_1 - T_2)}$	3937.17	2478.52	Schnute 1981; Baker et al. 1991
Seasonal	$L_2 = L_1 + (A - L_1) \cdot (1 - e^{-K \cdot D + S(t_1) - S(t_2)})$	**3609.63**	**2352.95**	Somers 1988

Note: AIC, Akaike's information criterion; *A*, asymptotic body size (mean ± SE); *K*, characteristic growth constant (day–1; mean ± SE); *L*1, snout–vent length (SVL) at time *i*; *L*2, SVL at time *i* + 1; *D*, days between *i* and *i* + 1; *y*1, minimum size observed at a given age (*T*1); *y*2, maximum sizes observed in the sample at a given age (*T*2). *S*(*ti*) = (*C* · *K*/2_) · sin[2_/365 · (*ti* − *ts*)], where *i* = 1 or 2, *t*1 is the time at first capture, *t*2 is the time at recapture (*t*2 = *t*1 + *D*), *C* is the proportion of decrease in growth (days; mean ± SE), and *ts* is the time shift for the annual cycle (days; mean ± SE). The retained model is in boldface type.

Materials and methods

Study area and species

Podarcis lilfordi is a medium-sized lizard endemic to the Balearic archipelago. Formerly found on the islands of Mallorca and Menorca, human-introduced predators have relegated the species to surrounding islets of the archipelago, prompting its listing as Endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) (Pérez-Mellado and Martínez-Solano 2009). Males are typically 10% larger than females, but morphological traits, including mean body size, can vary greatly from one population to another (Salvador 1980, 1986). Even though *P. lilfordi* is active all year round, lizards seem to reduce their daily activity during the coldest months of the year. The mating period is notwell defined, but it is thought to go from the beginning of the spring to the end of the summer, with the peak of births occurring around June (Pérez-Mellado and Salvador 1988; Castilla and Bauwens 2000). We used capture–recapture data collected on a 5 ha islet of the southern coast of

Mallorca (Moltona; 39°18'17.3''N, 03°00'43.2''E) between June 2009 and April 2015 over 16 sessions separated by a minimum of 60 days. In each session, animals were captured for three consecutive days on a grid of 47 pit-fall traps positioned along paths and vegetation edges, encompassing an area of 0.23 ha (more details in Ruiz de Infante Anton et al. 2014). Each captured lizard was measured from snout to vent (SVL) to the nearest millimetre and photographed for individual identification based on the unique pattern of pectoral scales (Perera and Perez-Mellado 2004; Sacchi et al. 2010) using the software APHIS (Moya et al. 2015). Adult lizards were sexed by visual inspection of sexually dimorphic characters (Dellinger and von Hegel 1990). Juveniles and subadults, whose secondary sexual characters are not clearly differentiated, have been sexed by counting the number of ventral-row scales (Salvador 1980; Lecomte et al. 1992; Le Galliard et al. 2005). To do this, we used photographs of the ventral region of a subset of adult lizards (185 males and 76 females) unequivocally sexed by secondary sexual characters to assess the number of scale rows of each sex. Females have a mean of 1–2 scale rows more than males (for more information see Supplementary material and Figs. S1 and S21). By this method, we were able to sex almost 75% of the 303 previously unsexed lizards. The remaining unsexed individuals were excluded from the analyses.

Growth patterns and length-at-age curve

We assessed which model adequately described the length-stage curve using individual data of SVL over time. To do this, we included positive and negative growth data because measurement errors might occur in both directions; furthermore, individuals can shrink in size due to senescence or in response to extreme resource limitation (Wikelski and Thom 2000; Smith et al. 2010). We considered four asymptotic growth equations to describe the size changes of individuals of unknown age (Table 1). The first two equations, the logistic-by-length model (Schoener and Schoener 1978) and von Bertalanffy's growth function (Fabens 1965;

Schoener and Schoener 1978), are the classical models used to describe growth in vertebrates (Lorenzen 1996; Katsanevakis and Maravelias 2008; Tsai et al. 2014). We also considered two additional models, Schnute's equation (Schnute 1981) and the oscillatory model (Somers 1988) (Table 1), which are different formulations of von Bertalanffy's growth function. In Schnute's equation, the main parameters of von Bertalanffy's model are expressed using the observed range of sizes to reduce their correlation. The oscillatory model incorporates a seasonal oscillation into von Bertalanffy's equation (Somers 1988), with a parameter C to modulate the amplitude of the growth so that if $C = 0$, then the equation reverts to the standard von Bertalanffy's growth function, and a parameter ts to set the time at which oscillations begin. This latter parameter also defines the winter point, which expresses the period of time when individuals grow more slowly (Pauly et al. 1992). The four models were implemented using a nonlinear mixed-effects model ("nlme" package; Pinheiro et al. 2008) in the R statistical package (R Core Team 2013). We considered the individual to be a random effect and included sex as a covariate in each parameter. We used Akaike's information criterion (AIC) to select the most parsimonious of the four growth models (Burnham and Anderson 2004). Following Pinheiro and Bates (2000), we performed diagnostic tests to assess heteroscedasticity of residuals and over-parameterization of random effects in the retained model.

Predictors of growth rate variation

The continuous growth curves described above were used to estimate the correct length-at-age curve, but these models cannot be used to assess the temporal variation in the growth rate because (*i*) the correlation among model parameters is high (Pilling 2002) and (*ii*) individuals might respond differently according to their age. Consequently, we sorted male and female lizards into three age classes according to the retained

length-at-age curve: juveniles (0–1 year), subadults (1–2 years), and adults (>2 years) (see a similar approach in Galán 1999; Le Galliard et al. 2010).

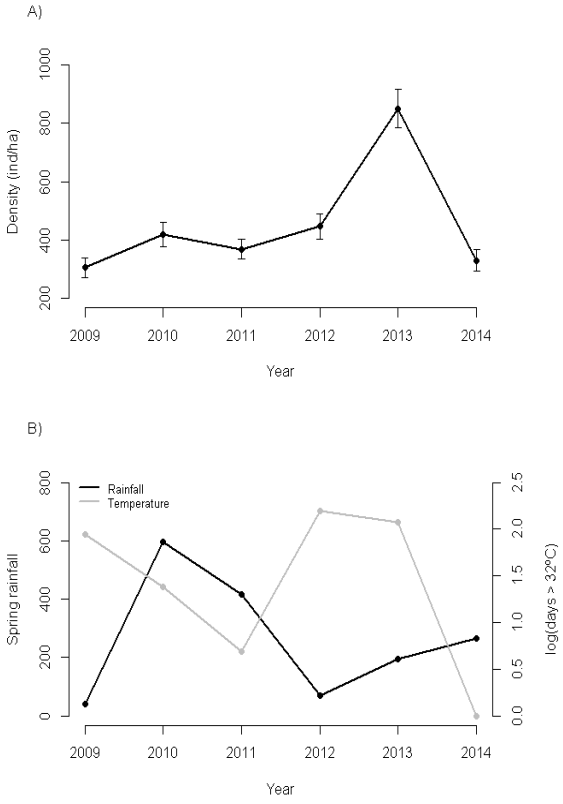

Fig. 1. Predictors for body growth of Lilford's Wall Lizard (*Podarcis lilfordi*). (A) Estimated lizard population size in April (except in 2009 when it was June) with associated SE based on the results from the Spatially Explicit Capture Recapture model (see text for details). (B) Rainfall (cumulative rainfall in May and June) and temperature (number of days with maximum temperature equal or greater than 32 °C).

In each age class, we calculated the individual growth rate and assessed whether it varied according to sex, year, and their statistical interaction using GLM with normal error structure (Nelder 1972). When yearly

variation proved to be significant, we assessed the effect of two covariates as predictors of temporal variability: the spring population size (see below) and the cumulative spring rainfall (May and June) (Figs. 1A, 1B). In addition, because Mediterranean climate is characterized by hot and dry summers, we took the log-transformed number of days in summer (from June to August) with a maximum temperature equal or more than 32 °C as an indication of harsh condition (Van Damme et al. 1991). Total lizard density, i.e., including all sizes, was estimated in April, except in 2009 when it was estimated during the first week of June, using Spatially Explicit Capture Recapture models (Efford 2004). This model uses capture–recapture data collected with an array of detectors (traps) to estimate population density and is based on a distance-dependent detection function of recapture, with mean g_0 and variance (Efford 2004). We used a common detection function across all sessions and modelled the parameters as a function of sex, time, and their interaction as in Ruiz de Infante *et al.* (2014). We included sex as a predictor of detection probability to ensure that males and females had different capture probability. During the 3-day session, the population was assumed to be closed, i.e., no births, deaths, immigration, or emigration occurred. Monte Carlo goodness-of-fit test was performed from the best model; this approach calculated a statistic from the model and related this statistic to the distribution of the statistic under the fitted model estimating the distribution with 99 simulation data from the model. Model selection followed the AIC criterion (see above; Burnham and Anderson 2004). Climatic variables during 2009 to 2014 were taken by a meteorological station at 5 km from the study area and provided by the Spanish State Meteorological Agency (available from http://www.aemet.es/es/portada) (Fig. 1B). The amount of variation accounted for by the covariates was calculated as [DEV(*c*) – DEV(.)]/[DEV(*t*) – DEV(.)], where DEV(*t*) is the deviance of the full-time dependent model, DEV(.) is the deviance of a model with no effects, and DEV(*c*) was the model assuming an effect of a given covariate (Harris et al. 2005). This measure (hereafter D^2)

corresponds to the proportion of explained variation and was comparable with a squared correlation coefficient (Schemper 1990).

Results

Growth patterns and length-at-age curve

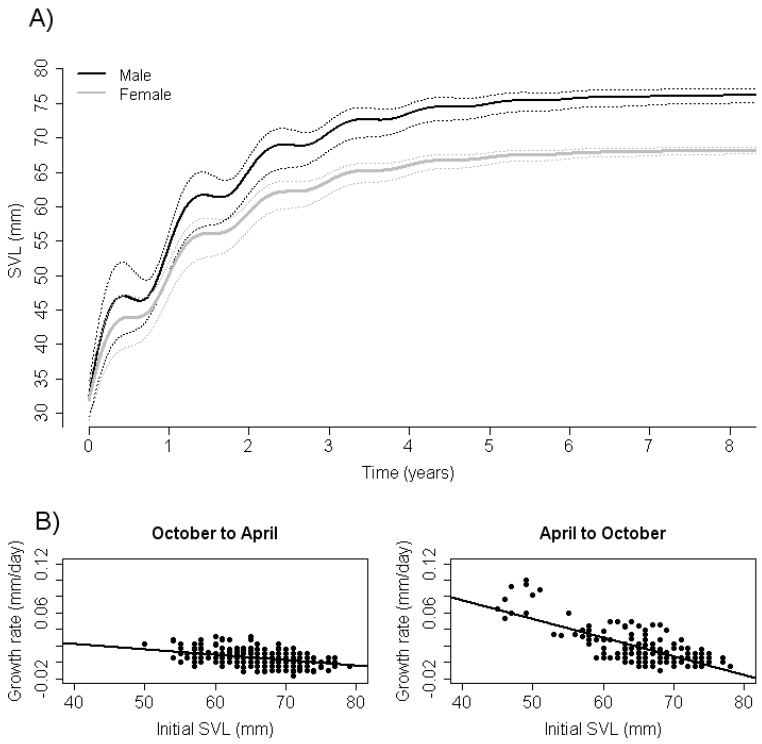

Fig. 2. (A) Estimated seasonal growth in males and females of Lilford's Wall Lizard (*Podarcis lilfordi*). (B) Growth rate versus initial snout–vent length (SVL) from winter (October to April) and summer (April to October).

Body size growth was modelled using 1496 observations from 586 lizards (345 males and 241 females) captured at least twice. The AIC values indicated that the oscillatory model (fourth equation) was the best model

to describe the growth of both males and females (Table 1). Given this result, we combined data into a single analysis and assessed whether growth parameters were sex specific using model information theory. The modelling of the joint data indicated that males have a significantly larger asymptotic size than females (A_{males} = 76.32 ± 0.26, $A_{females}$ = 68.33 ± 0.20; t = 29.68, $p < 0.001$), but both sexes had similar values of the characteristic growth parameter K (K_{males} = 0.002 ± 0.00007, $K_{females}$ = 0.0019 ± 0.00008; t = 1.15, p = 0.2513). Although they presented a statistically significant difference, both sexes shared similar values that reflect the intensity of the seasonal growth oscillation (C) (C_{males} = 1.2 ± 0.12, $C_{females}$ = 1.01 ± 0.10; t = 2.13, p = 0.033), whereas the time when sinusoid growth begins (ts) (ts-males = –0.46 ± 0.03, ts-females = –0.54 ± 0.03; t = –0.30, p = 0.76) was the same for both sexes. Therefore, we reduced this general model by assuming a shared growth constant and the starting point of oscillation in both sexes, but no further simplifications of the model structure were possible. According to our final model, parameter estimates (mean ± SE) were as follows: A_{males} = 76.76 ± 0.33, $A_{females}$ = 68.69 ± 0.26; K = 0.0019 ± 0.00014; C_{males} = 1.2 ± 0.13, $C_{females}$ = 1 ± 0.11; ts = –0.48 ± 0.03. Estimates of A in this final model were still statistically different between the sexes (t = 24.03, $p < 0.001$), with only K as a random effect. Expected growth trajectories from a 31.9 mm SVL hatchling (Castilla and Bauwens 2000) to a hypothetical maximum age of 10 years showed no overlap between males and females (Fig. 2A). The estimated size-at-age growth curves indicated that males and females differed in size from hatchling as growth trajectories diverge at the first moment (Fig. 2A). The amplitude of growth oscillation (C) is virtually 1 in both sexes, suggesting that growth increased by 100% during spring–autumn and becomes 0 during the winter. This result is supported by the observed growth data recorded in the two seasons (n_{winter} = 335 observations, n_{summer} = 417 observations; Fig. 2B). Following the retained growth curve, we estimated that juveniles (0–1 year) ranged from 31.9 to 58mmfor males and from 31.9 to 53 mm for females, yearlings (1–2 years) ranged from 59 to 67mmfor males and from

54 to 62 for females, and larger lizards were considered adults (2+ years; SLV >68 mm for males and SVL >63 mm for females).

Table 2. Akaike's information criterion (AIC) values of models assuming the effect of year and sex on the growth rate of juvenile, yearling, and adult Lilford's Wall Lizards (*Podarcis lilfordi*).

Model	No. of parameters	Juvenile	Yearling	Adult
Year × sex	10	327.03	773.73	1344.51
Year + sex	6	**322.95**	**768.42**	1340.45
Year	5	327.92	776.36	1345.27
Sex	2	335.52	785.72	**1337.71**
Null	1	335.25	793.53	1342.81

Note: × denotes the presence of a statistical interaction between main effects, whereas + denotes its absence, i.e., additive relationship. Best models are in boldface type. Because in adult lizards the effect of year was not retained, a possible effect of covariates was not evaluated.

Predictors of growth rate variation

The oscillatory model indicated that lizards did not growth during the winter; consequently, we only considered the growth rate from April to October and modelled its variation within each age-by-sex class as a function of population size and climatic variables (see below). As expected, the growth rate abated with age (juveniles = 0.066 ± 0.024 mm/day, yearlings = 0.027 ± 0.015 mm/day, adults = 0.006 ± 0.010 mm/day; ANOVA: $F_{[2,414]} = 312.6$, $p < 0.0001$). Yearly variation was retained in juveniles and yearlings only with an additive difference between males and females (Table 2). In these two age classes, we assessed how much of this variation was explained by intrinsic and extrinsic factors (Table 3). In juveniles, we found a significant effect of density ($F_{[1,43]} = 14.92$, $p = 0.0004$), rainfall ($F_{[1,43]} = 4.12$, $p = 0.05$), and temperature ($F_{[1,43]} = 10.37$, $p = 0.002$). For juveniles, while density and temperature had a statistically significant negative influence on growth rate, the cumulative rainfall had a

positive one (Figs. 3A–3C). Note that the negative relationship holds even when the extreme value of 2013 was not considered (results not shown). In yearlings, the only variable that influenced the growth rate was the cumulative rainfall in spring (density: $F_{[1,116]} = 0.001$, $p = 0.98$; rainfall: $F_{[1,116]} = 10.49$, $p = 0.002$; temperature: $F_{[1,116]} = 0.014$, $p = 0.9$; Figs. 3D–3F).

Table 3. Assessing the influence of population density (D), spring rainfall (R), and summer temperature (T) in explaining the yearly variation in the growth rate of juvenile and yearling Lilford's Wall Lizards (*Podarcis lilfordi*).

		Juvenile		Yearling	
Model	No. of parameters	AIC	D^2	AIC	D^2
Year	7	322.95	1	768.42	1
D + T + R	6	319.64	0.98	775.12	0.74
D	3	**320.74**	0.81	787.72	0.30
R	3	**331.51**	0.39	**775.60**	0.62
T	3	**326.33**	0.60	787.23	0.31
Null	1	335.25	0	793.54	0

Note: All models assumed an additive effect of sex. Because in adult lizards the effect of year was not retained, a possible effect of covariates was not evaluated. AIC, Akaike's information criterion; *D*2, proportion of the temporal deviance explained by the covariate (see text). Significant effects are in boldface type ($p < 0.05$).

Discussion

In vertebrates, the length-at-age curve is thought to be shaped by the optimal allocation of energy between fertility and somatic growth or somatic maintenance (Charnov 1993). Consequently, a description of growth patterns and the factors influencing them can provide an important insight into an individual's life-history tactics and demography (Caley and Schwarzkopf 2004; Laver et al. 2012; Tsai et al. 2014). Here we contrasted different growth models to select the most appropriate length-at-age curve for *P. lilfordi*. As expected, we found a different growth pattern for males and females, likely because of different energy

allocations (Shine 1990; Heino and Kaitala 1999; Wikelski and Thom 2000). Males had the same growth parameter (*K*) as females, but differed in the asymptotic maximum size, which suggest a faster growth (Figs. 2A, 2B). Consequently, males reached sexual maturity at a similar age and matured at a larger size than females. Although this pattern is observed in many other *Podarcis* species (Bocage's Wall Lizard, *Podarcis bocagei* (Seoane, 1884): Galán 1999; Milos Wall Lizard, *Podarcis milensis* (Bedriaga, 1882): Adamopoulou and Valakos 2000; Carbonell's Wall Lizard, *Podarcis carbonelli* Pérez-Mellado, 1981: Almeida et al. 2001; Common Wall Lizard, *Podarcis muralis* (Laurenti, 1768): Bauwens and Díaz-Uriarte 1997), the common pattern observed in many reptiles is a positive correlation in sex differences between sexual size maturity and asymptotic maximum size (Trivers 1976; Schoener and Schoener 1978; Gibbons and Lovich 1990;Stamps 1993).

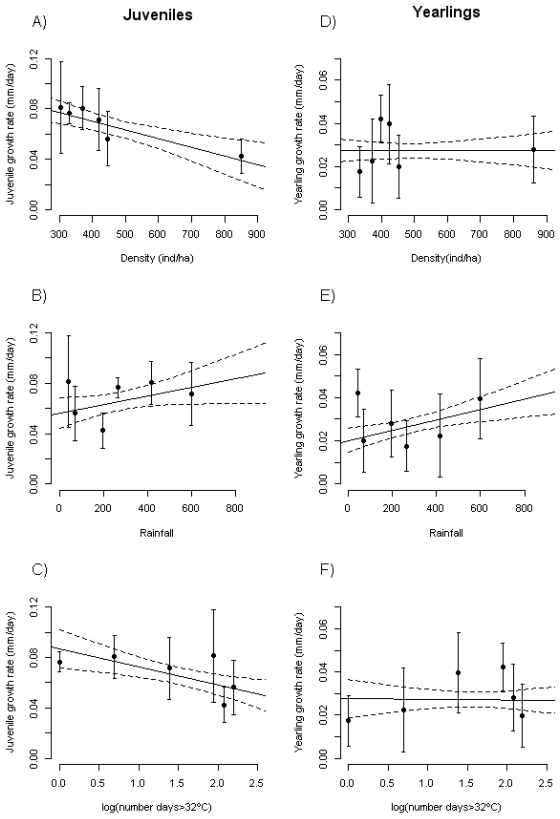

Fig. 3. Variation in body growth rates of juvenile (A, B, C) and yearling (D, E, F) Lilford's Wall Lizards (*Podarcis lilfordi*) according to population density and climatic variables. In juveniles, relationships were all statistically significant, whereas only rainfall seemed to influence growth rate of yearlings (E; see text for details).

A common *K* value for males and females indicates that environmental factors affecting the growth of males have similar effects on females (Stamps 1993). Moreover, the selected model included an oscillatory term, suggesting periods of no or very slow growth (Fig. 2B). The growth of most plant species and ectothermic animals is strongly seasonal (Pauly 1990; Alcoverro et al. 1995; Coma et al. 2000), but there are very few studies on growth of reptiles that take into account a seasonality pattern (but see Adolph and Porter 1996; Smith et al. 2010). *Podarcis lilfordi*, like other lizard species that share similar climatic condition, seldom

hibernates (Pérez-Mellado and Salvador 1981; Pérez-Mellado 1982; Galán 1997). Salvador (1986) reported that *P. lilfordi* reduces the daily activity period in the coldest months of the year and it is reasonable to think that food intake is too low to maintain a fast body growth. Moreover, the winter point near zero indicated the importance of per-capita resource availability during the spring–summer period, something we further investigated by sorting males and females into three age classes based on their respective length-at-age curve. In large vertebrates, density dependent and independent factors have been shown to influence many life-history traits, but their relative role differed according to individual age or sex (Van Valen 1965; Andrews 1976; Ballinger and Congdon 1980; Massot et al. 1992; Coulson et al. 2001; Le Galliard et al. 2010; Mugabo et al. 2010, 2011, 2013). In small vertebrates, such a fine description of the role of density and climatic factors on multiple life-history traits is difficult to obtain in natural populations. Evidence for lizards comes from artificial or seminatural populations, and the effect of density and climatic conditions has mainly been studied considering these factors separately (Andrews 1976; Le Galliard et al. 2010; Mugabo et al. 2010, 2011, 2013). Using long-term individual-based data, we were able to analyze these factors simultaneously and estimate their relative importance according to age and sex. We showed that the relative role of density and climatic factors in modulating the individual growth rate changed according to the age considered and in a similar way for males and females (Table 3). Furthermore, insular conditions are likely to intensify the effects of these factors because insular populations are typically very dense. Adults (2+ years) were not noticeably affected by either density or climatic factors. For adult males, this is probably the result of despotic competition for resources and their social dominance over smaller lizards (Massot et al. 1992; Lecomte et al. 1994; Mugabo et al. 2010). However, we found that growth rate was also constant in adult females, suggesting that intersexual competition does not affect somatic growth of females. Massot et al. (1992) suggested that body size in females is maintained to a

detriment of their reproductive success. This seems like a plausible explanation; because it has been shown that growth was negatively linked to reproduction in reptiles (Laurie and Brown 1990) and more research should be done to further investigate this point. Contrary to adults, the growth of juvenile lizards was very variable over time. This temporal variability was negatively associated with population density and summer temperature, but positively associated with spring rainfall. This is in accordance with asymmetric trophic and social interactions because adult lizards are dominant over yearlings and juveniles (Pilorge 1988). The level of despotic competition can be high in particular periods of resource availability (Pérez-Mellado et al. 2015) and the reported cannibalism in insular population is its extreme degree (Castilla and van Damme 1996; Cooper et al. 2015). Otherwise, the level of competition is thus likely to be mediated by the interaction between density and food availability as experimentally demonstrated by the positive link between association between per-capita food intake and growth rate (Le Galliard et al. 2005). Dunham (1978) also found hatchings that emerged in years with high rainfall had rapid growth rates. In juveniles, the effect of density was stronger than the one of the other covariates, explaining about 80% of the temporal variability in growth rate. Only 17% of the variability was explained by climatic variables. These results contrasted with those found in yearlings, as their growth rate was not influenced by population density and the only statistically significant predictor was the cumulative spring rainfall (positive effect). This agrees with Tinkle et al.'s (1993) study that reported body sizes attained by yearling Common Sagebrush Lizards (*Sceloporus graciosus* Baird and Girard, 1852) were positively correlated with annual rainfall values. The effect of rainfall is likely to be indirect, as rainfall improves habitat humidity, plant growth, and insect productivity, thereby increasing the food intake of lizards (Stamps and Tanaka 1981; Madsen and Shine 2000). This is consistent with what we found for juveniles and yearlings, thus confirming the importance of water constraints in temperate lizards (Lorenzon et al. 1999; Le Galliard et al.

2010). We found a negative effect of temperature on body growth of juveniles. It is known that thermal requirements are crucial for the thermoregulation and energy balance of reptiles (see Lourdais et al. 2008). Although temperature variation among years was low, we expected a negative correlation between the number of hot days and the lizard's body growth. Van Damme et al. (1991) further showed the mass of Viviparous Lizards (*Zootoca vivipara* (Jacquin, 1787), formerly *Lacerta vivipara* Jacquin, 1787) were reduced when the temperature reached 35 °C. Therefore, the more days with high temperatures, the less growing lizards will have. This association was found in juveniles only, being the most sensitive to climate factors. Thermoregulation in juveniles is not good, yet it is possible that physiological functions experience a decline when temperature reaches the thermal maxima (Sunday et al. 2011; Hoffmann et al. 2013). The limited number of years hampered us from testing more complex relationships between growth rate and predictors. We cannot exclude nonlinear relationships or a possible role of the interaction between density and climatic variables. Despite these limitations, our results revealed the oscillatory pattern or growth and suggested a complex interplay between extrinsic and intrinsic factors on the variability of body growth rate. Their different roles according to individual stage (age or size) predict a complex pattern at the population level, considering that current climatic conditions will always have a delayed effect on future recruitments. Le Gaillard et al. (2010) suggested that an environmental-dependent growth rate was the mechanism underlying the differences among cohorts. Our results support this view and suggest a more complex pattern because stochastic events, e.g., the alternation of wet and dry summers, can generate compensatory responses, e.g., a low growth during the juvenile stage can be partially compensated for by a faster growth at a yearling stage. Given a density-dependent body growth of juvenile and a climatic, but density-independent, driven growth of yearlings, it is not surprising to find a high heterogeneity of body sizes within the population (Figs. 2A, 2B). Further

research is needed to assess the demographic consequence of this complexity.

Acknowledgements

We are grateful to members of the Population Ecology Group at IMEDEA and also to M. Palmer of the Fish Ecology Group at IMEDEA for their help in all phases of this work. We thank the Port at Colonia St. Jordi, Guillermo from "Excursion Boat", and L. Bonnet for their logistic support, and the Conselleria de Medi Ambient of the Balearic government for permission to handle animals. We are grateful to S. Baille for his help with the oscillatory model. We specially thank C. Adamopolou, A. Castillo, and P. Pafilis for all their support. A.R. was supported by a JAE fellowship from the Spanish Research Council (CSIC, Spain).

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Appendix A

Adult Balearic lizards (*Podarcis lilfordi)* can be sexed at capture by visual inspection (Dellinger et al. 1990). However, we were not always able to sex juveniles and sub-adults lizards reliably (but see Arnold and Burton 1978) . To illustrate this problem we selected a subset of 56 adult males and 35 adult females whose sex was determined with certainty and that was previously captured at least once as juveniles (<50 mm). We used the sex assigned at each capture to calculate the probability to correctly determining the sex by visual inspection as a function of SVL. As expected there was a positive correlation between SVL and the probability of correctly assigning sex with a 0.5 probability was predicted at 56.5 mm in females and 62.5 mm in males (Fig. A1). One way to solve this problem and be able to sex most individuals is counting ventral scale rows. In *Podarcis lilfordi* Salvador (1980) showed that females have on average more ventral scale rows than males but, the difference changed across populations. We sexed our study population and males of *P. lilfordi* in Moltona had on average fewer ventral scale rows than females ($F_{1,332}$ = 328.4, $p < 0.001$). Lizards with more than 28 scale rows were always females and those with fewer than 26 were always males (Fig. A2). Longitudinal analyses indicated that scale number remained constant through the individual's lifespan. Individuals that were not sexed by external characteristics and presented between 26 and 28 scale rows were not included in the analysis.

Figure A1. Probability of correctly assigning sex in function of the individual's size.

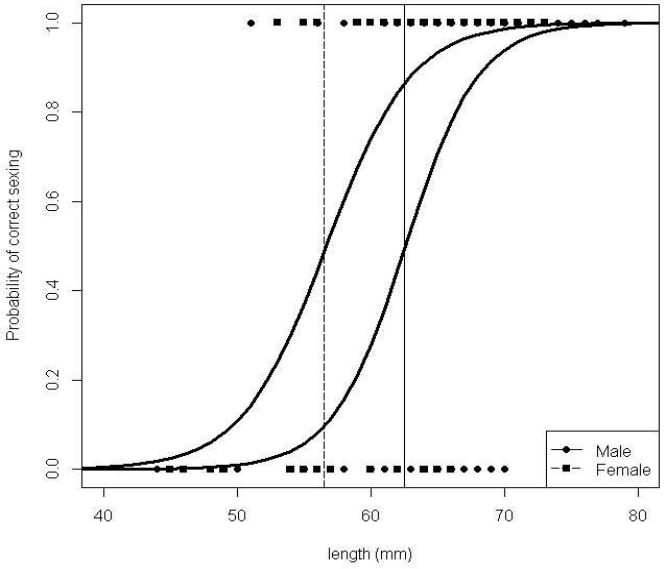
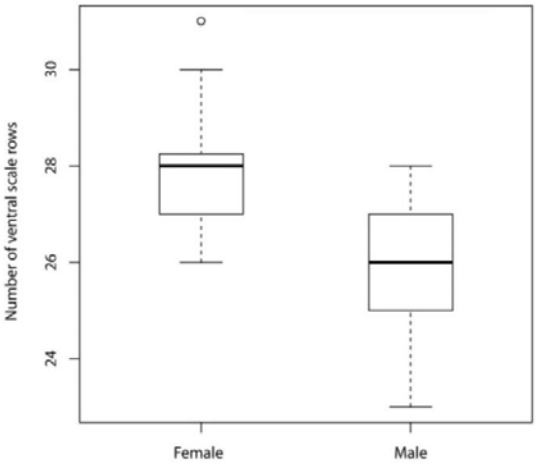


Figure A2. Number of ventral scale rows in adult lizards (*Podarcis lilfordi*) of known sex.



APPENDIX

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ARTICLE

Relative role of population density and climatic factors in shaping the body growth rate of Lilford's Wall Lizard (*Podarcis lilfordi*)

A. Rotger, J.M. Igual, J.J. Smith, and G. Tavecchia

Abstract: The body growth rate in small reptiles is modulated by per-capita food resources and recent evidences suggested that this constraint is the mechanism underlying differences between cohorts. Per-capita food resources depend on population size and climatic factors, but their relative role in explaining the variations in growth rate is unclear. We used morphological data collected over 6 years to model the body growth of an insular lizard (Lilford's Wall Lizard, *Podarcis lilfordi* (Günther, 1874)). We used nonlinear equations to describe the appropriate length-at-age relationship. For each sex, seasonal growth was observed and the oscillatory von Bertalanffy curve was fitted to the data. Three age classes were recognized, and we investigated the relative role of density, spring rainfall, and temperature in explaining the variation of the growth rate in each sex-by-age class. Results showed that the relative role of density and climatic factors varied according to the age considered. While population size and temperature had a negative effect on the growth rate of juveniles, rainfall had a positive influence on the growth of subadults. Adult growth was near zero and constant over time. The different role of density-dependent and climatic factors in explaining age-dependent growth rate provides an important insight in understanding lizard population dynamics and life-history tactics.

Key words: density-dependent growth, climate, capture–recapture, oscillatory growth, age classes, Balearic Islands, Lilford's Wall Lizard, *Podarcis lilfordi*.

Résumé : Le taux de croissance du corps chez les petits reptiles est modulé par les ressources alimentaires par individu, et des observations récentes donnent à penser que cette contrainte est le mécanisme qui sous-tend des différences entre cohortes. Les ressources alimentaires par individu dépendent de la taille de la population et de facteurs climatiques, mais l'incidence relative de ces différents facteurs sur les variations des taux de croissance n'est pas bien établie. Nous avons utilisé des données morphologiques recueillies sur une période de six ans pour modéliser la croissance corporelle d'un lézard insulaire (le lézard de Lilford, *Podarcis lilfordi* (Günther, 1874)). Nous avons utilisé des équations non linéaires pour décrire la relation de la longueur selon l'âge pertinente. Pour chacun des sexes, la croissance saisonnière a été observée et la distribution oscillatoire de von Bertalanffy a été calée sur les données. Trois classes d'âge ont été détectées, et nous avons examiné les rôles relatifs de la densité, des pluies printanières et de la température pour expliquer les variations du taux de croissance dans chaque classe de sexe selon l'âge. Les résultats montrent que les incidences relatives de la densité et des facteurs climatiques varient selon l'âge. Si la taille de la population et la température ont un effet négatif sur le taux de croissance des juvéniles, la pluie a une influence positive sur la croissance des sous-adultes. La croissance des adultes est presque nulle et constante dans le temps. Ces différents rôles de facteurs dépendant de la densité et de facteurs climatiques pour expliquer le taux de croissance selon l'âge jettent un nouvel éclairage important sur la dynamique des populations de lézards et les tactiques associées à leurs cycles biologiques. [Traduit par la Rédaction]

Mots-clés : croissance dépendant de la densité, climat, capture–recapture, croissance oscillatoire, classes d'âge, îles Baléares, lézard de Lilford, *Podarcis lilfordi*.

Introduction

In species with continuous growth, the rate at which individuals attain the size of sexual maturity represents an important life-history trait and is involved in multiple evolutionary trade-offs (Stearns 1992; Caley and Schwarzkopf 2004; Laver et al. 2012). Large variation in growth rate across individuals and populations may result from differences in genetic components (Niewiarowski and Roosenburg 1993), food availability (Dunham 1978), environmental factors, and their interactions (Sinervo and Adolph 1989; Angilletta et al. 2004). However, inter- and intra-population variations in growth suggest a high degree of phenotypic plasticity (Stamps et al. 1998; Badyaev 2002; Sears and Angilletta 2004), and the realized body size-at-age relationship is not only the result of evolutionary links, but also of this complex interplay between local resource abundance (Van Valen 1965; Hentschel 1999) and environmental conditions (Schoener and Schoener 1978; Stamps 1993). Despite these two factors being expected to concurrently influence individual growth rate, their role has been usually assessed separately (Le Gaillard et al. 2010; Mugabo et al. 2013). Consequently, the relative importance of population size and climatic factors on body growth rate is often unclear (but see Henle et al. 2004; Zabel et al. 2006). Moreover, the effects of density-dependent and independent factors can vary according to

Received 11 September 2015. Accepted 22 December 2015.

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Table 1. Growth models for individuals of unknown age.

Model	Equation	AIC		References
		Males	Females	
Logistic	$L_2 = A \cdot L_1/[L_1 + (A - L_1)e^{-K \cdot D}]$	3983.23	2529.34	Schoener and Schoener 1978
von Bertalanffy	$L_2 = A - (A - L_1)e^{-K \cdot D}$	3964.31	2508.54	Fabens 1965
Schnute†	$L_2 = L_1 \cdot e^{-K \cdot D} + (y_2 - y_1 \cdot e^{-K(T_1 - T_2)}) \cdot 1 - e^{-K \cdot D}/1 - e^{-K \cdot (T_1 - T_2)}$	3937.17	2478.52	Schnute 1981; Baker et al. 1991
Seasonal	$L_2 = L_1 + (A - L_1) \cdot (1 - e^{-K \cdot D + S(t_1) - S(t_2)})$	**3609.63**	**2352.95**	Somers 1988

Note: AIC, Akaike's information criterion; A, asymptotic body size (mean ± SE); K, characteristic growth constant (day^{-1}; mean ± SE); L_1, snout–vent length (SVL) at time i; L_2, SVL at time $i + 1$; D, days between i and $i + 1$; y_1, minimum size observed at a given age (T_1); y_2, maximum sizes observed in the sample at a given age (T_2). $S(t_i) = (C \cdot K/2\pi) \cdot \sin[2\pi/365 \cdot (t_i - t_s)]$, where $i = 1$ or 2, t_1 is the time at first capture, t_2 is the time at recapture ($t_2 = t_1 + D$), C is the proportion of decrease in growth (days; mean ± SE), and t_s is the time shift for the annual cycle (days; mean ± SE). The retained model is in boldface type.

individual sex or age (e.g., Massot et al. 1992; Grant and Benton 2000; Coulson et al. 2001). In lizards, for example, the sexes play different roles in reproduction and typically have divergent growth that results in different adult sizes, i.e., sexual size dimorphism (Badyaev 2002). In general terms, female body size and growth rate is shaped by selective pressures on fecundity, whereas male size and growth rate is thought to be driven largely by male–male competition (Taylor and DeNardo 2005). Stage-specific and sex-specific life-history data are therefore often needed to produce realistic dynamics of structured populations regulated by density and climatic fluctuations (Coulson et al. 2001; Zabel et al. 2006). Here, we used individual-based data to fit the length-at-age curve of the endemic Balearic wall lizard (Lilford's Wall Lizard, *Podarcis lilfordi* (Günther, 1874)) from capture–recapture data of uniquely identified individuals. Once the length-at-age relationship was described, we quantified the relative role of population size and climatic factors affecting the body growth rate of juvenile, subadults, and adult lizards. Overall, insular populations of lizards attain a density that is 10 times higher than the mainland counterparts (Buckley and Jetz 2007). This high density, promoted by the lack of predators (Buckley and Jetz 2007; Pérez-Mellado et al. 2008), is expected to limit individual growth rate because per-capita resources are low (Adler and Levins 1994; Grant 1998). Consequently, a first expectation is that individual growth is negatively related to population size (Le Gaillard et al. 2010). Besides the common features of insular populations, the Mediterranean environment (characterized by dry summers and relatively mild winters) imposes different constraints from those found in oceanic climate (Pérez-Mellado and Corti 1993). The high summer temperatures limit resource availability in islands, promoting herbivory, despotic competition, and in the extreme cases, cannibalism (Pérez-Mellado and Traveset 1999; Cooper et al. 2015; Pérez-Cembranos and Pérez-Mellado 2015; Pérez-Mellado et al. 2015). Thus, climatic conditions such as rainfall and temperature are expected to be positively associated with individual growth rate through their influence on habitat quality and lizard physiological processes, respectively (Lorenzon et al. 1999; Jordan and Snell 2002; Marquis et al. 2008). We thus predicted a difference in the growth rate of males and females due to sexual differences in physiology, morphology, and behavior (Le Galliard et al. 2005, 2010). We assessed these predictions using individual data collected over 6 years on individually recognized lizards of known sex.

Materials and methods

Study area and species

Podarcis lilfordi is a medium-sized lizard endemic to the Balearic archipelago. Formerly found on the islands of Mallorca and Menorca, human-introduced predators have relegated the species to surrounding islets of the archipelago, prompting its listing as Endangered by the International Union for Conservation of Nature and Natural Resources (IUCN) (Pérez-Mellado and Martínez-Solano 2009). Males are typically 10% larger than females, but morphological traits, including mean body size, can vary greatly from one population to another (Salvador 1980, 1986). Even though *P. lilfordi* is active all year round, lizards seem to reduce their daily activity during the coldest months of the year. The mating period is not well defined, but it is thought to go from the beginning of the spring to the end of the summer, with the peak of births occurring around June (Pérez-Mellado and Salvador 1988; Castilla and Bauwens 2000). We used capture–recapture data collected on a 5 ha islet of the southern coast of Mallorca (Moltona; 39°18′17.3″N, 03°00′43.2″E) between June 2009 and April 2015 over 16 sessions separated by a minimum of 60 days. In each session, animals were captured for three consecutive days on a grid of 47 pit-fall traps positioned along paths and vegetation edges, encompassing an area of 0.23 ha (more details in Ruiz de Infante Anton et al. 2014). Each captured lizard was measured from snout to vent (SVL) to the nearest millimetre and photographed for individual identification based on the unique pattern of pectoral scales (Perera and Perez-Mellado 2004; Sacchi et al. 2010) using the software APHIS (Moya et al. 2015). Adult lizards were sexed by visual inspection of sexually dimorphic characters (Dellinger and von Hegel 1990). Juveniles and subadults, whose secondary sexual characters are not clearly differentiated, have been sexed by counting the number of ventral-row scales (Salvador 1980; Lecomte et al. 1992; Le Galliard et al. 2005). To do this, we used photographs of the ventral region of a subset of adult lizards (185 males and 76 females) unequivocally sexed by secondary sexual characters to assess the number of scale rows of each sex. Females have a mean of 1–2 scale rows more than males (for more information see Supplementary material and Figs. S1 and S2[1]). By this method, we were able to sex almost 75% of the 303 previously unsexed lizards. The remaining unsexed individuals were excluded from the analyses.

Growth patterns and length-at-age curve

We assessed which model adequately described the length-at-age curve using individual data of SVL over time. To do this, we included positive and negative growth data because measurement errors might occur in both directions; furthermore, individuals can shrink in size due to senescence or in response to extreme resource limitation (Wikelski and Thom 2000; Smith et al. 2010). We considered four asymptotic growth equations to describe the size changes of individuals of unknown age (Table 1). The first two equations, the logistic-by-length model (Schoener and Schoener 1978) and von Bertalanffy's growth function (Fabens 1965; Schoener and Schoener 1978), are the classical models used to describe growth in vertebrates (Lorenzen 1996; Katsanevakis and Maravelias

[1]Supplementary material and figures are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2015-0188.

2008; Tsai et al. 2014). We also considered two additional models, Schnute's equation (Schnute 1981) and the oscillatory model (Somers 1988) (Table 1), which are different formulations of von Bertalanffy's growth function. In Schnute's equation, the main parameters of von Bertalanffy's model are expressed using the observed range of sizes to reduce their correlation. The oscillatory model incorporates a seasonal oscillation into von Bertalanffy's equation (Somers 1988), with a parameter C to modulate the amplitude of the growth so that if $C = 0$, then the equation reverts to the standard von Bertalanffy's growth function, and a parameter t_s to set the time at which oscillations begin. This latter parameter also defines the winter point, which expresses the period of time when individuals grow more slowly (Pauly et al. 1992). The four models were implemented using a nonlinear mixed-effects model ("nlme" package; Pinheiro et al. 2008) in the R statistical package (R Core Team 2013). We considered the individual to be a random effect and included sex as a covariate in each parameter. We used Akaike's information criterion (AIC) to select the most parsimonious of the four growth models (Burnham and Anderson 2004). Following Pinheiro and Bates (2000), we performed diagnostic tests to assess heteroscedasticity of residuals and over-parameterization of random effects in the retained model.

Predictors of growth rate variation

The continuous growth curves described above were used to estimate the correct length-at-age curve, but these models cannot be used to assess the temporal variation in the growth rate because (*i*) the correlation among model parameters is high (Pilling 2002) and (*ii*) individuals might respond differently according to their age. Consequently, we sorted male and female lizards into three age classes according to the retained length-at-age curve: juveniles (0–1 year), subadults (1–2 years), and adults (>2 years) (see a similar approach in Galán 1999; Le Galliard et al. 2010). In each age class, we calculated the individual growth rate and assessed whether it varied according to sex, year, and their statistical interaction using GLM with normal error structure (Nelder 1972). When yearly variation proved to be significant, we assessed the effect of two covariates as predictors of temporal variability: the spring population size (see below) and the cumulative spring rainfall (May and June) (Figs. 1A, 1B). In addition, because Mediterranean climate is characterized by hot and dry summers, we took the log-transformed number of days in summer (from June to August) with a maximum temperature equal or more than 32 °C as an indication of harsh condition (Van Damme et al. 1991). Total lizard density, i.e., including all sizes, was estimated in April, except in 2009 when it was estimated during the first week of June, using Spatially Explicit Capture Recapture models (Efford 2004). This model uses capture–recapture data collected with an array of detectors (traps) to estimate population density and is based on a distance-dependent detection function of recapture, with mean g_0 and variance σ (Efford 2004). We used a common detection function across all sessions and modelled the parameters as a function of sex, time, and their interaction as in Ruiz de Infante et al. (2014). We included sex as a predictor of detection probability to ensure that males and females had different capture probability. During the 3 day session, the population was assumed to be closed, i.e., no births, deaths, immigration, or emigration occurred. Monte Carlo goodness-of-fit test was performed from the best model; this approach calculated a statistic from the model and related this statistic to the distribution of the statistic under the fitted model estimating the distribution with 99 simulation data from the model. Model selection followed the AIC criterion (see above; Burnham and Anderson 2004). Climatic variables during 2009 to 2014 were taken by a meteorological station at 5 km from the study area and provided by the Spanish State Meteorological Agency (available from http://www.aemet.es/es/portada) (Fig. 1B). The amount of variation accounted for by the covariates was calculated as [DEV(c) – DEV(.)]/[DEV(t) – DEV(.)], where DEV(t) is the deviance of the full-time dependent model, DEV(.) is the deviance of a model with no effects, and DEV(c) was the model assuming an effect of a given covariate (Harris et al. 2005). This measure (hereafter D^2) corresponds to the proportion of explained variation and was comparable with a squared correlation coefficient (Schemper 1990).

Results

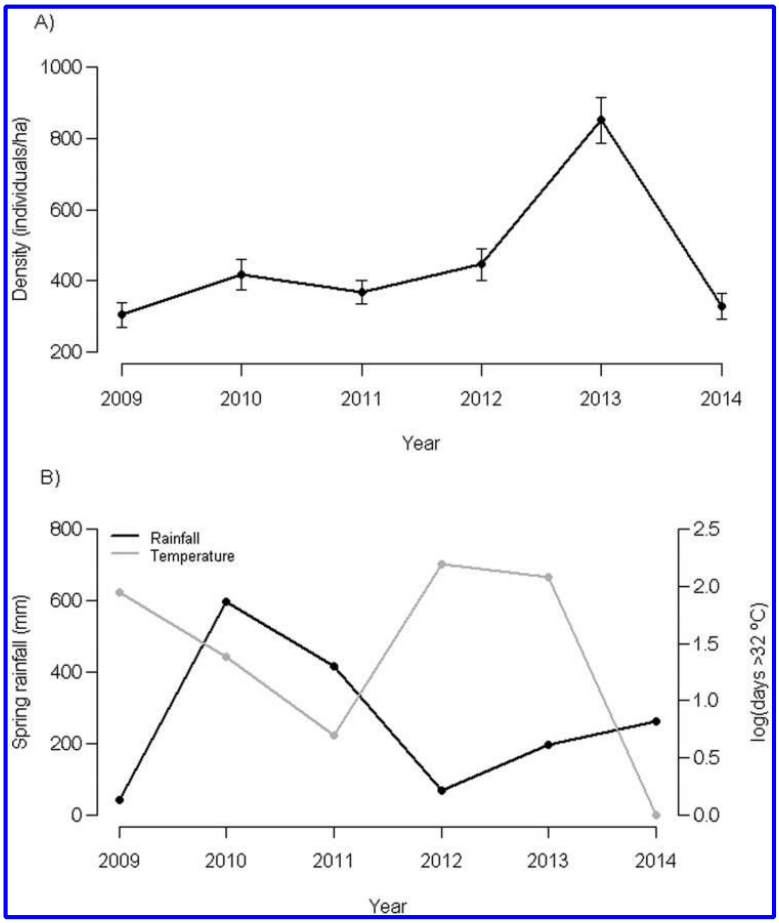
Growth patterns and length-at-age curve

Body size growth was modelled using 1496 observations from 586 lizards (345 males and 241 females) captured at least twice. The AIC values indicated that the oscillatory model (fourth equation) was the best model to describe the growth of both males and females (Table 1). Given this result, we combined data into a single analysis and assessed whether growth parameters were sex-specific using model information theory. The modelling of the joint data indicated that males have a significantly larger asymptotic size than females ($A_{males} = 76.32 \pm 0.26$, $A_{females} = 68.33 \pm 0.20$; $t = 29.68$, $p < 0.001$), but both sexes had similar values of the characteristic growth parameter K ($K_{males} = 0.002 \pm 0.00007$, $K_{females} = 0.0019 \pm 0.00008$; $t = 1.15$, $p = 0.2513$). Although they presented a statistically significant difference, both sexes shared similar values that reflect the intensity of the seasonal growth oscillation (C) ($C_{males} = 1.2 \pm 0.12$, $C_{females} = 1.01 \pm 0.10$; $t = 2.13$, $p = 0.033$), whereas the time when sinusoid growth begins (t_s) ($t_{s\text{-males}} = -0.46 \pm 0.03$, $t_{s\text{-females}} = -0.54 \pm 0.03$; $t = -0.30$, $p = 0.76$) was the same for both sexes. Therefore, we reduced this general model by assuming a shared growth constant and the starting point of oscillation in both sexes, but no further simplifications of the model structure were possible. According to our final model, parameter estimates (mean ± SE) were as follows: $A_{males} = 76.76 \pm 0.33$, $A_{females} = 68.69 \pm 0.26$; $K = 0.0019 \pm 0.00014$; $C_{males} = 1.2 \pm 0.13$, $C_{females} = 1 \pm 0.11$; $ts = -0.48 \pm 0.03$. Estimates of A in this final model were still statistically different between the sexes ($t = 24.03$, $p < 0.001$), with only K as a random effect. Expected growth trajectories from a 31.9 mm SVL hatchling (Castilla and Bauwens 2000) to a hypothetical maximum age of 10 years showed no overlap between males and females (Fig. 2A). The estimated size-at-age growth curves indicated that males and females differed in size from hatchling as growth trajectories diverge at the first moment (Fig. 2A). The amplitude of growth oscillation (C) is virtually 1 in both sexes, suggesting that growth increased by 100% during spring–autumn and becomes 0 during the winter. This result is supported by the observed growth data recorded in the two seasons ($n_{winter} = 335$ observations, $n_{summer} = 417$ observations; Fig. 2B). Following the retained growth curve, we estimated that juveniles (0–1 year) ranged from 31.9 to 58 mm for males and from 31.9 to 53 mm for females, yearlings (1–2 years) ranged from 59 to 67 mm for males and from 54 to 62 for females, and larger lizards were considered adults (2+ years; SLV >68 mm for males and SVL >63 mm for females).

Predictors of growth rate variation

The oscillatory model indicated that lizards did not growth during the winter; consequently, we only considered the growth rate from April to October and modelled its variation within each age-by-sex class as a function of population size and climatic variables (see below). As expected, the growth rate abated with age (juveniles = 0.066 ± 0.024 mm/day, yearlings = 0.027 ± 0.015 mm/day, adults = 0.006 ± 0.010 mm/day; ANOVA: $F_{[2,414]} = 312.6$, $p < 0.0001$). Yearly variation was retained in juveniles and yearlings only with an additive difference between males and females (Table 2). In these two age classes, we assessed how much of this variation was explained by intrinsic and extrinsic factors (Table 3). In juveniles, we found a significant effect of density ($F_{[1,43]} = 14.92$, $p = 0.0004$), rainfall ($F_{[1,43]} = 4.12$, $p = 0.05$), and temperature ($F_{[1,43]} = 10.37$, $p = 0.002$). For juveniles, while density and temperature had a

Fig. 1. Predictors for body growth of Lilford's Wall Lizard (*Podarcis lilfordi*). (A) Estimated lizard population size in April (except in 2009 when it was June) with associated SE based on the results from the Spatially Explicit Capture Recapture model (see text for details). (B) Rainfall (cumulative rainfall in May and June) and temperature (number of days with maximum temperature equal or greater than 32 °C).



statistically significant negative influence on growth rate, the cumulative rainfall had a positive one (Figs. 3A–3C). Note that the negative relationship holds even when the extreme value of 2013 was not considered (results not shown). In yearlings, the only variable that influenced the growth rate was the cumulative rainfall in spring (density: $F_{[1,116]} = 0.001$, $p = 0.98$; rainfall: $F_{[1,116]} = 10.49$, $p = 0.002$; temperature: $F_{[1,116]} = 0.014$, $p = 0.9$; Figs. 3D–3F).

Discussion

In vertebrates, the length-at-age curve is thought to be shaped by the optimal allocation of energy between fertility and somatic growth or somatic maintenance (Charnov 1993). Consequently, a description of growth patterns and the factors influencing them can provide an important insight into an individual's life-history tactics and demography (Caley and Schwarzkopf 2004; Laver et al. 2012; Tsai et al. 2014). Here we contrasted different growth models to select the most appropriate length-at-age curve for *P. lilfordi*. As expected, we found a different growth pattern for males and females, likely because of different energy allocations (Shine 1990; Heino and Kaitala 1999; Wikelski and Thom 2000). Males had the same growth parameter (K) as females, but differed in the asymptotic maximum size, which suggest a faster growth (Figs. 2A, 2B). Consequently, males reached sexual maturity at a similar age and matured at a larger size than females. Although this pattern is observed in many other *Podarcis* species (Bocage's Wall Lizard, *Podarcis bocagei* (Seoane, 1884): Galán 1999; Milos Wall Lizard, *Podarcis milensis* (Bedriaga, 1882): Adamopoulou and Valakos 2000; Carbonell's Wall Lizard, *Podarcis carbonelli* Pérez-Mellado, 1981: Almeida et al. 2001; Common Wall Lizard, *Podarcis muralis* (Laurenti, 1768): Bauwens and Díaz-Uriarte 1997), the common pattern observed in many reptiles is a positive correlation in sex differences between sexual size maturity and asymptotic maximum size (Trivers 1976; Schoener and Schoener 1978; Gibbons and Lovich 1990; Stamps 1993). A common K value for males and females indicates that environmental factors affecting the growth of males have similar effects on females (Stamps 1993). Moreover, the selected model included an oscillatory term, suggesting periods of no or very slow growth (Fig. 2B). The growth of most plant species and ectothermic animals is strongly seasonal (Pauly 1990; Alcoverro et al. 1995; Coma et al. 2000), but there are very few

Fig. 2. (A) Estimated seasonal growth in males and females of Lilford's Wall Lizard (*Podarcis lilfordi*). (B) Growth rate versus initial snout–vent length (SVL) from winter (October to April) and summer (April to October).

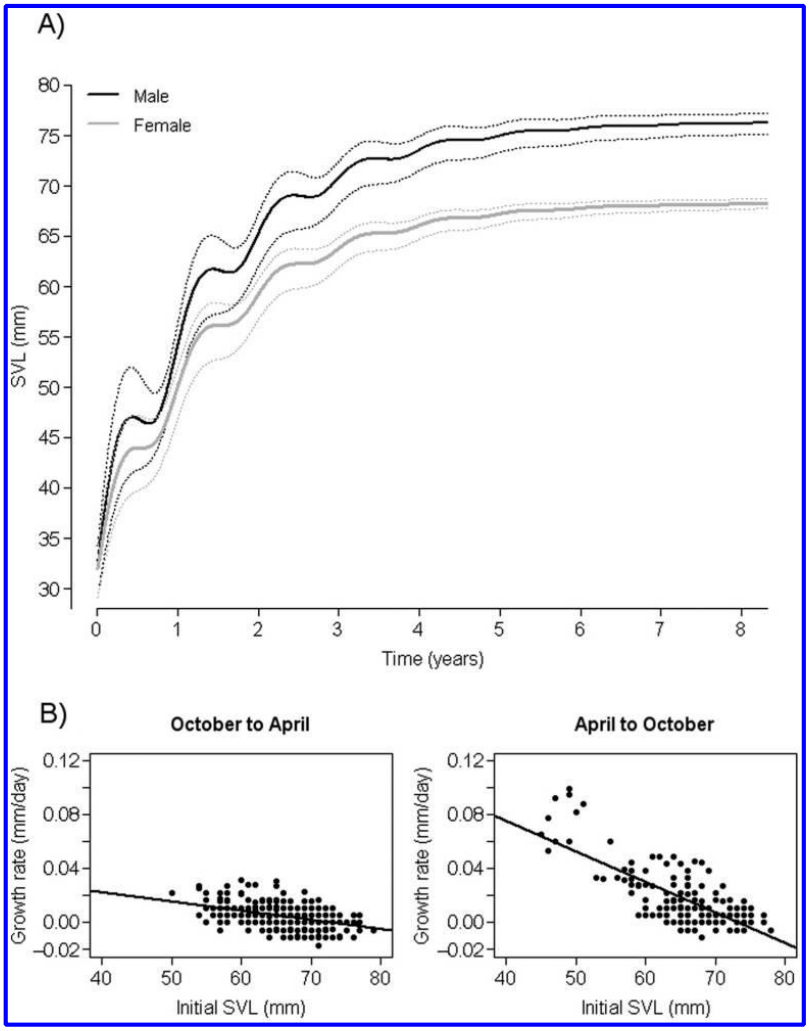


Table 2. Akaike's information criterion (AIC) values of models assuming the effect of year and sex on the growth rate of juvenile, yearling, and adult Lilford's Wall Lizards (*Podarcis lilfordi*).

Model	No. of parameters	Juvenile	Yearling	Adult
Year × sex	10	327.03	773.73	1344.51
Year + sex	6	**322.95**	**768.42**	1340.45
Year	5	327.92	776.36	1345.27
Sex	2	335.52	785.72	**1337.71**
Null	1	335.25	793.53	1342.81

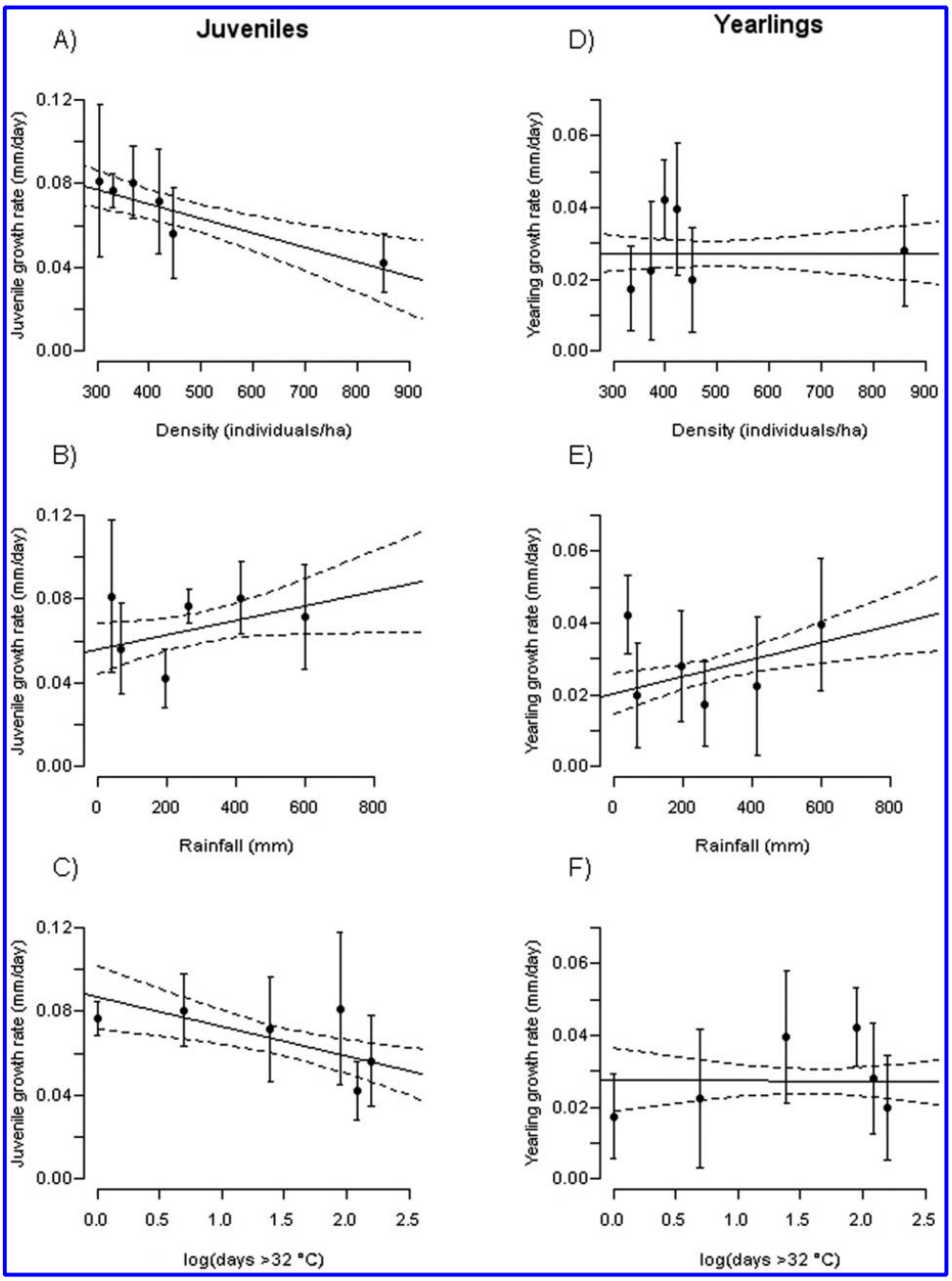
Note: × denotes the presence of a statistical interaction between main effects, whereas + denotes its absence, i.e., additive relationship. Best models are in boldface type. Because in adult lizards the effect of year was not retained, a possible effect of covariates was not evaluated.

Table 3. Assessing the influence of population density (D), spring rainfall (R), and summer temperature (T) in explaining the yearly variation in the growth rate of juvenile and yearling Lilford's Wall Lizards (*Podarcis lilfordi*).

		Juvenile		Yearling	
Model	No. of parameters	AIC	D^2	AIC	D^2
Year	7	322.95	1	768.42	1
D + T + R	6	319.64	0.98	775.12	0.74
D	3	**320.74**	0.81	787.72	0.30
R	3	**331.51**	0.39	**775.60**	0.62
T	3	**326.33**	0.60	787.23	0.31
Null	1	335.25	0	793.54	0

Note: All models assumed an additive effect of sex. Because in adult lizards the effect of year was not retained, a possible effect of covariates was not evaluated. AIC, Akaike's information criterion; D^2, proportion of the temporal deviance explained by the covariate (see text). Significant effects are in boldface type ($p < 0.05$).

Fig. 3. Variation in body growth rates of juvenile (A, B, C) and yearling (D, E, F) Lilford's Wall Lizards (*Podarcis lilfordi*) according to population density and climatic variables. In juveniles, relationships were all statistically significant, whereas only rainfall seemed to influence growth rate of yearlings (E; see text for details).



studies on growth of reptiles that take into account a seasonality pattern (but see Adolph and Porter 1996; Smith et al. 2010). *Podarcis lilfordi*, like other lizard species that share similar climatic condition, seldom hibernates (Pérez-Mellado and Salvador 1981; Pérez-Mellado 1982; Galán 1997). Salvador (1986) reported that *P. lilfordi* reduces the daily activity period in the coldest months of the year and it is reasonable to think that food intake is too low to maintain a fast body growth. Moreover, the winter point near zero indicated the importance of per-capita resource availability during the spring–summer period, something we further investigated by sorting males and females into three age classes based on their respective length-at-age curve. In large vertebrates, density-dependent and independent factors have been shown to influence many life-history traits, but their relative role differed according to individual age or sex (Van Valen 1965; Andrews 1976; Ballinger and Congdon 1980; Massot et al. 1992; Coulson et al. 2001; Le Galliard et al. 2010; Mugabo et al. 2010, 2011, 2013). In small vertebrates, such a fine description of the role of density and climatic factors on multiple life-history traits is difficult to obtain in natural populations. Evidence for lizards come from artificial or seminatural populations, and the effect of density and climatic conditions has mainly been studied considering these factors separately (Andrews 1976; Le Galliard et al. 2010; Mugabo et al. 2010, 2011, 2013). Using long-term individual-based data, we were able to analyze these factors simultaneously and estimate their relative importance according to age and sex. We showed that the relative role of density and climatic factors in modulating the individual growth rate changed according to the age considered and in a similar way for males and females (Table 3). Furthermore, insular conditions are likely to intensify the effects of these factors because insular populations are typically very dense. Adults (2+ years) were not noticeably affected by either density or climatic factors. For adult males, this is probably the result of despotic competition for resources and their social dominance over smaller lizards (Massot et al. 1992; Lecomte et al. 1994; Mugabo et al. 2010). However, we found that growth rate was also constant in adult females, suggesting that intersexual competition does not affect somatic growth of females. Massot et al. (1992) suggested that body size in females is maintained to a detriment of their reproductive success. This seems like a plausible explanation, because it has been shown that growth was negatively linked to reproduction in reptiles (Laurie and Brown 1990) and more research should be done to further investigate this point. Contrary to adults, the growth of juvenile lizards was very variable over time. This temporal variability was negatively associated with population density and summer temperature, but positively associated with spring rainfall. This is in accordance with asymmetric trophic and social interactions because adult lizards are dominant over yearlings and juveniles (Pilorge 1988). The level of despotic competition can be high in particular periods of resource availability (Pérez-Mellado et al. 2015) and the reported cannibalism in insular population is its extreme degree (Castilla and van Damme 1996; Cooper et al. 2015). Otherwise, the level of competition is thus likely to be mediated by the interaction between density and food availability as experimentally demonstrated by the positive link between association between per-capita food intake and growth rate (Le Galliard et al. 2005). Dunham (1978) also found hatchings that emerged in years with high rainfall had rapid growth rates. In juveniles, the effect of density was stronger than the one of the other covariates, explaining about 80% of the temporal variability in growth rate. Only 17% of the variability was explained by climatic variables. These results contrasted with those found in yearlings, as their growth rate was not influenced by population density and the only statistically significant predictor was the cumulative spring rainfall (positive effect). This agrees with Tinkle et al.'s (1993) study that reported body sizes attained by yearling Common Sagebrush Lizards (*Sceloporus graciosus* Baird and Girard, 1852) were positively correlated with annual rainfall values. The effect of rainfall is likely to be indirect, as rainfall improves habitat humidity, plant growth, and insect productivity, thereby increasing the food intake of lizards (Stamps and Tanaka 1981; Madsen and Shine 2000). This is consistent with what we found for juveniles and yearlings, thus confirming the importance of water constraints in temperate lizards (Lorenzon et al. 1999; Le Galliard et al. 2010). We found a negative effect of temperature on body growth of juveniles. It is known that thermal requirements are crucial for the thermoregulation and energy balance of reptiles (see Lourdais et al. 2008). Although temperature variation among years was low, we expected a negative correlation between the number of hot days and the lizard's body growth. Van Damme et al. (1991) further showed the mass of Viviparous Lizards (*Zootoca vivipara* (Jacquin, 1787), formerly *Lacerta vivipara* Jacquin, 1787) were reduced when the temperature reached 35 °C. Therefore, the more days with high temperatures, the less growing lizards will have. This association was found in juveniles only, being the most sensitive to climate factors. Thermoregulation in juveniles is not good, yet it is possible that physiological functions experience a decline when temperature reaches the thermal maxima (Sunday et al. 2011; Hoffmann et al. 2013). The limited number of years hampered us from testing more complex relationships between growth rate and predictors. We cannot exclude nonlinear relationships or a possible role of the interaction between density and climatic variables. Despite these limitations, our results revealed the oscillatory pattern or growth and suggested a complex interplay between extrinsic and intrinsic factors on the variability of body growth rate. Their different roles according to individual stage (age or size) predict a complex pattern at the population level, considering that current climatic conditions will always have a delayed effect on future recruitments. Le Gaillard et al. (2010) suggested that an environmental-dependent growth rate was the mechanism underlying the differences among cohorts. Our results support this view and suggest a more complex pattern because stochastic events, e.g., the alternation of wet and dry summers, can generate compensatory responses, e.g., a low growth during the juvenile stage can be partially compensated for by a faster growth at a yearling stage. Given a density-dependent body growth of juvenile and a climatic, but density-independent, driven growth of yearlings, it is not surprising to find a high heterogeneity of body sizes within the population (Figs. 2A, 2B). Further research is needed to assess the demographic consequence of this complexity.

Acknowledgements

We are grateful to members of the Population Ecology Group at IMEDEA and also to M. Palmer of the Fish Ecology Group at IMEDEA for their help in all phases of this work. We thank the Port at Colonia St. Jordi, Guillermo from "Excursion Boat", and L. Bonnet for their logistic support, and the Conselleria de Medi Ambient of the Balearic government for permission to handle animals. We are grateful to S. Baille for his help with the oscillatory model. We specially thank C. Adamopolou, A. Castillo, and P. Pafilis for all their support. A.R. was supported by a JAE fellowship from the Spanish Research Council (CSIC, Spain).

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Chapter III

Contrasting life-histories in neighbouring populations of lizards: the role of ancient human settlement.

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Photo source: G. Tavecchia

Contrasting life-histories in neighbouring populations of lizards: the role of ancient human settlement.

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Abstract

Lizards can exhibit marked differences in body size across populations that at large spatial scale are generally in agreement with thermoregulatory constraints as predicted by the Bergmann's rule. However, differences across neighbouring populations cannot be ascribed to climate. We considered three close, predatory-free, island populations of the Lilford's lizard *Podarcis lilfordi* with differences from 2% to 20% in the realized adult body size. We coupled genetic, morphometric, ecological and demographic information to infer the forces responsible for these spatial differences. Neither genetic divergence, nor islet size, nor islet biotic capacity index, nor population density matches the morphological differences. Moreover, individual growth was slow in the smallest island, where lizards reached the largest average body size suggesting that resource availability does not constrain realized body size. In agreement with the hypothesis of human-related pressure against larger body size, the smallest lizards were found in the only islet inhabited by humans in ancient times (400-200BC). Bayesian computation (ABC) analyses of the genetic data indicated a bottleneck in this lizard population corresponding to the date of the human settlement.

Also lizard average survival, reproduction and size at first reproduction are similar to those found in continental populations in presence of predators. Despite the evidence for an effect of past human settlement against large body sizes is only indirect, we emphasize the need to integrate ecological and genetic data and the importance of considering past human colonization as an additional force modelling present island fauna.

Key-words: body size, bottleneck, individual-based data, insular lizards, microevolution.

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Resum

Les sargantanes poden presentar marcades diferències en la grandària del cos a poblacions que, en gran escala espacial, estan en general en concordança amb les limitacions de termoregulació segons el predit per la regla de Bergmann. No obstant això, les diferències entre les poblacions veïnes no poden atribuir-se al clima.

Es van considerar 3 poblacions insulars properes de sargantana balear (*Podarcis lilfordi)* amb diferències poblacionals d'entre el 2% al 20% en la grandària final del cos adult. Es varen agrupar segons informació morfològica, genètica, ecològica i demogràfica per inferir amb les forces responsables d'aquestes diferències espacials. Ni la divergència genètica, ni la mida dels illots, ni l'índex de capacitat biòtica de l'illot, ni la densitat de població coincideixen amb les diferències morfològiques. D'altra banda, el creixement individual era lent a l'illa més petita, on les sargantanes van aconseguir la major mitjana de mesura del cos, el que suggereix que la disponibilitat de recursos no limita la mida del cos. D'acord amb la hipòtesi de la pressió humana en contra de la grandària més gran, les sargantanes més petites van ser trobades a l'únic illot habitat en l'antiguitat (400-200BC). L'anàlisi bayesià (ABC) de les dades genètiques indica un coll d'ampolla en aquesta població de sargantanes que correspon a la data de l'assentament humà. També la mitjana de supervivència de la sargantana, reproducció i talla de primera reproducció són similars als trobats en les poblacions continentals en presència de depredadors. Tot i que l'evidència de l'efecte dels assentaments humans en el passat contra grans mides corporals és només indirecta, fem èmfasi en la necessitat d'integrar les dades ecològiques i genètiques i la importància de considerar la colonització humana passada com un agent addicional modalitzador de la fauna insular present.

Paraules clau: Mida corporal, dades del individu, sargantanes insulars, microevolució.

Introduction

The differences in adult body size across vertebrate's populations have interested bio-geographers and evolutionary ecologists for decades (Peters, 1986, Begon et al. 1990). The two main forces responsible for spatial differences are site-specific selective forces and random genetic processes, i.e. genetic drift. Changes caused by adaptive responses are typically more rapid than those imposed by random processes and when trait plasticity is high they might occur without genetic changes (Reznick *et al*. 2004; Clegg *et al*. 2008; Schoener 2011). In ectotherms, changes across populations can be ascribed to thermoregulatory strategies and, at a large spatial scale, body size differences are associated with latitude and elevation as predicted by the Bergman's rule (Aragón & Fitze 2014). However, body size might also be different between neighbouring populations sharing the same climate due to different levels of per-capita resources (Wikelski & Trillmich, 1997; McNab, 2010) or predatory pressure (Bassar *et al*. 2013). A particular type of predatory pressure responsible for body size shifts is the one derived by human activities (see also Oro et al. 2013). Harvesting, for example, has been shown to be responsible for a reduction in body size and an earlier onset of the reproduction in fish (Hutchings 2004). As harvesting it is commonly directed toward larger body sizes (Hutchings 2004; Law 2007), it is expected to have evolutionary consequences (Coltman *et al*. 2003; Fenberg & Roy 2008). Human-caused body size shift can also be caused indirectly by adaptations to human-modified biotic and abiotic environment, i.e. habitat loss or introduced species (Palumbi 2001; Hendry, Farrugia & Kinnison 2008). Pregill (1986) showed that even indirect human-related selective pressures can cause evolutionary changes in body size. In small islands inhabited by humans, small-medium size lizards became smaller as a response to the introduced species and the modified habitats. Despite human activities have provided many evidences about the rapid evolutionary responses of wild populations in many taxa (Ratner & Lande 2001; Olsen *et al*. 2004; Walsh

et al. 2006; Proaktor, Coulson & Milner-Gulland 2007), human-related selective pressures are seldom considered to explain spatial differences in body size and their evolutionary and demographic consequences remain unclear (Walsh et al., 2006, but see(Proaktor *et al*. 2007; Allendorf *et al*. 2008). Recently isolated populations, such as those on continental islands, offer a good biological model to try to separate the relative contribution of these evolutionary forces because evolutionary processes occur on contemporary timescales (Blondel *et al*. 1991; Thompson 1998; Schluter 2000; Stockwell *et al*. 2003; Whittaker & Fernández-Palacios 2007; Clegg 2010). Whereas genetic drift is likely play an important role in new island populations, adaptive responses may be important when populations colonize habitats with specific selective pressures (but see (Campbell & Echternacht 2003; Velo-Antón *et al*. 2012).

We considered the body size differences in three geographically close islets inhabited by the endemic Balearic wall lizard (*Podarcis lilfordi*, Günther, 1874). Islets have been separated from each other over 4000-5000 years ago, therefore the three populations are likely to derive from the same recent colonization (Berg, M.P. van den 2015). Indeed, a study based on differences in the mitochondrial DNA has showed that the three isolated populations belong to the same genetic clade with the supposed population of origin at the island of Cabrera Grande (Terrasa *et al*. 2009). The islets are predatory free and inhabited by a single lizard species, however, one of the islets was colonised by a Punic settlement in 400 BC that lasted about 200 years (Guerrero 1997, 2000). Islets today differ in size and plant composition but have a comparable, yet not identical, density of lizards (de Infante Anton *et al*. 2014). We investigated the role of human settlement, ecological conditions and genetic differences in explaining the phenotypic changes. In addition, because body size changes can affect other life-history traits we measured adult survival, fertility and body growth in all three islets using experimental data and individual-based data collected into the field. If per-capita resources

influenced body size, we predict the smallest lizard to inhabit the smallest and/or more densely populated islet. On the other hand if human settlement induced an adaptive shift in body sizes we should find the smallest lizards on the once inhabited islet, which should also show an excess of heterozygosity and a low number of private alleles due to the past selection.

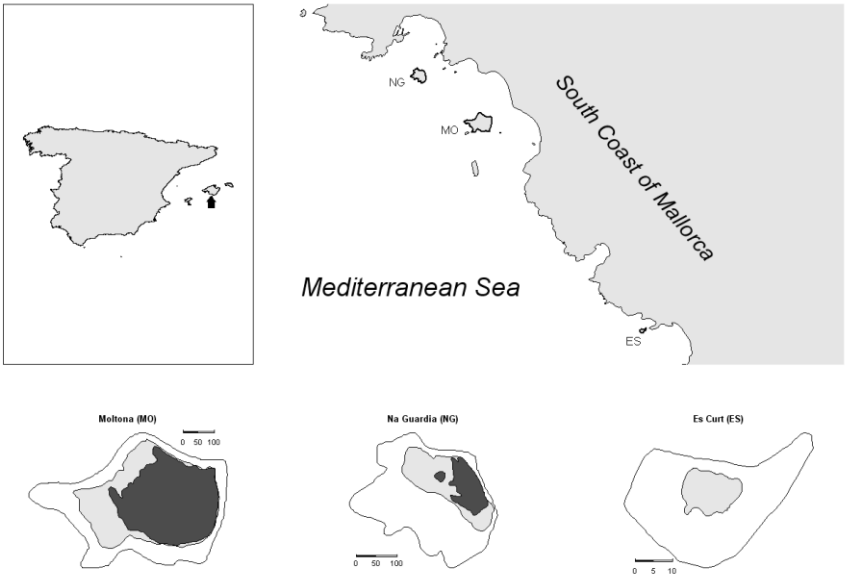

Figure 1. Location and vegetation structure of the three lizard populations. Coastline, rocky and dunes community (white), herbaceous community (light gray), shrub community (dark gray).

Material and methods

Study area and ecological characteristics

We studied three islets of the southern coast of Mallorca: Na Moltona (MO, hereafter), Na Guardia (NG) and Es Curt (ES; Fig. 1). We updated

the available floristic checklist by Rita and Bibiloni (2003) and described the vegetation assembly of each islet in terms of number of species present and number of plants with fleshy fruits. Data and islet characteristics and plant assembly were used to describe the ecological setting of each islet. We calculated the index D of biotic capacity (Cheylan 1992) as $D_i = \log(S_i \times a_i)$, where '$S_i$' is the surface of the *i*-islet and 'a' is its maximal altitude (Parlanti *et al*. 1988; Pérez-Mellado *et al*. 2008).

Table 1. Physical and biotic characteristics of the three study populations.

	Moltona (MO)	Na Guardia (NG)	Es Curt (ES)
Area (ha)*	5.09	1.98	0.29
Maximum altitude (m)	11	9	5
Distance to main island (m)	350	300	30
Distance to the nearest islet (m)	900	900	3500
Historical human settlement	No	Yes	No
Seabird nests (since year 2006)	Yes	Yes	Occasional
Biotic capacity index (D)*	4.03	2.45	0.37
Lizard density (ind/ha)**	1316	1560	2381
N. plant species	108	76	15
Area of shrub community **	2.7	0.26	0
Ruderals plants	32	22	4
Meadow flowering plants	28	15	0
sp. Fleshy fruit	12	8	3

*from Pérez-Mellado *et al*. 2008

** from spatially explicit capture-recapture models in Ruiz de Infante Anton *et al*. 2013

Genetic analysis

Sampling and molecular analysis: Genomic DNA was extracted from the tail tissue of 80 individuals (ES = 28, MO = 27 and NG= 25) using standard phenol–chloroform protocols. Fifteen specific microsatellites were considered (Bloor *et al*. 2011). PCRs were run in a GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Foster City, CA, USA), using primers and conditions described in Bloor et al. (2011). Fluorescently labelled PCR products were run on an ABI 3130 DNA Sequencer (Applied Biosystems) with GeneScan-500 (LIZ) internal size standard. Fragment length was assigned using GeneMapper software v3.2 (Applied Biosystems) and visually rechecked.

Genetic structure and gene flow: We used GENALEX V6.2 (Peakall & Smouse 2006) to calculate allele frequencies from standard measures of microsite diversity. We also measured genetic diversity, observed and expected heterozygosity, inbreeding coefficient (F_{IS}) and to assess deviations from the Hardy-Weinberg equilibrium. The procedure FreeNa was used to test the presence of null alleles for each locus using the ENA correction method (Chapuis & Estoup 2006). Population subdivision was first evaluated using Bayesian assignment tests in STRUCTURE v2.3 (Pritchard, Stephens & Donnelly 2000) to identify the number of distinct clusters (K). To estimate the number of genetically distinct clusters, ten independent runs assuming a ad-mixture model and correlated allele frequencies were carried out for each value between K= 1 to K=5 at 10^6 Markov Chain Monte Carlo (MCMC) repetitions and a burn-in period of 10^5 iterations. The number of distinct genetic clusters was determined through assessment of ΔK (Evanno, Regnaut & Goudet 2005) using STRUCTURE HARVESTER (Earl 2012). We complemented this analysis with a Principal Components Analysis (PCA) of the 15 microsatellite loci, using Adegenet package (Jombart 2008) in R 3.1.0 (R Development Core Team, 2008). Finally, to determine the degree of differentiation among genetic

clusters, we performed an analysis of molecular variance (AMOVA) and pairwise F_{ST} (Weir & Cockerham 1984) and R_{ST} (Slatkin 1995) values were calculated in GENALEX V6.2 (Peakall & Smouse 2006). The presence of a possible recent gene flow among islands (i.e. during the past one-three generations) was assessed using BayesAss (version 3.0; (Wilson & Rannala 2003). The algorithm was run for $50x10^6$ iterations with a burn-in period of 10^6 iterations and a sampling frequency of $2x10^3$ iterations. Our final run used delta-values of m = 0.15, a = 0.45 and f = 0.35, where 'm' is the migration rate, 'a' is the allele frequencies and 'f' is the inbreeding coefficient to yield an average number of changes in the accepted range between 40% and 60% of the total chain length (Wilson & Rannala 2003).

Demographic bottlenecks and population history (ABC analysis): The software BOTTLENECK (Piry, Luikart & Cornuet 1999) was used to test which populations may have undergone a significant reduction in size. We tested the presence of an excess of heterozygotes using Wilcoxon signed rank test under three mutation models; infinite alleles (IAM), two-phase (TPM), and stepwise mutation model (SMM). We ran all mutation models with 20,000 iterations, and for TPM we set ps = 0.9 (frequency of single step mutations) and the variance of those mutations as 12 (Busch, Waser & DeWOODY 2007). Finally, we applied M-ratio test to investigate a genetic bottleneck further in the past (> 100 generations) with software M_P_VAL (Garza & Williamson 2001). The analysis of genetic structure supported the separation of the three populations (see below) with ES being the most differentiated population, probably due to a process of isolation by distance (Wright 1943; Rousset 1997). We used Approximate Bayesian Computation analysis (ABC; (Cornuet *et al.* 2008) to infer populations history using microsatellite data (Lander *et al.* 2011). We compared three demographic scenarios (Fig. 3) that differed in when population might have diverged in the past at different times (t1 and t2 respectively measured in generation time), the number of populations present at each time step and the population size (N). Scenarios 1 is a null

model assuming separation of population did not cause changes in population size and did not affect the genetic pool. Scenario 2 assumed a past reduction in ES and the ancestral MO+NG population, a description of population divergences as suggested by the genetic structure. Finally, scenario 3 like scenario 2 simulated an additional bottleneck due to human settlement in NG that lasted 500-600 generations (Ayuso 1981). Models were compared by estimating their posterior probabilities using the logistic regression method (Ghirotto et al. 2010, see Additional Information).

Demographic traits

Capture-recapture data : Every October from 2010 to 2015 we conducted a three-day capture-recapture session in each islet (6 sessions). Individual lizards were caught in geo-referred pitfall traps positioned along paths and vegetation edges (see Ruiz de Infante *et al.* 2014). Each captured lizard was weighted, measured from snout to vent (SVL) to the nearest mm, photographed using a digital camera for individual identification (Moya et al. 2015) and subsequently released near the same trap of capture. Immature and adult lizards were sexed by the inspection of the femoral pores. Lizard less than 1-year old (c. SVL<50mm) were sexed by counting the number of row ventral scales (Lecomte et al. 1992, Rotger et al, 2016).

Individual growth analysis: We analysed growth for each sex-by-islet combination using capture recapture data from 1369 lizards (355 males and 243 females from MO, 276 males and 100 females from NG, 209 males and 189 females from ES) that have been captured at least twice. We estimated the characteristic growth rate (K) and the asymptotic size (y_2) using the parameterization proposed by Baker et al. (1991), i.e a modification of the Schnute's growth model for mark-recaptured data as in Rotger et al. (2016). The parameters K and size y_2 were modeled using individual as random effect to account for individual variability using

nonlinear mixed-effects model ("nlme" package; (Pinheiro et al.) 2008). We included population and sex as a factorial explanatory variables (population: 3 levels, MO, NG and ES; sex: 2 levels, male and females) and used Akaike's information criterion (AIC) to select the most parsimonious model (Burnham & Anderson 2004). Furthermore, we performed diagnostics tests to assess heterocedasticity of residuals and overparameterization of random effects (Pinheiro & Bates 2000).

Survival and fecundity analyses: we estimated mean October-to-October local survival probability (Φ) from 2010 to 2015 in males and females on the three islets. We used the encounter history of 1680 individuals (MO: 400 males and 316 females; NG: 362 males and 187 females; ES: 173 males and 242 females). We first assessed the goodness of fit of a model assuming survival and probability of recapture varying over time (Cormack-Jolly-Seber model, CJS) using the program U-care (Choquet *et al*. 2009). Survival and recapture probability were modelled as a function of time, sex, islet and their statistical interactions in program MARK (White & Burnham 1999). Estimates were obtained by maximum likelihood from the capture-recapture histories of individually recognized lizards (Burnham & Anderson, 2004; see Additional information for more detals). Models were compared using the corrected Akaike Information Criterion (AICc, (Anderson & Burnham 2002). Finally, to accommodate model selection uncertainty, we used model averaging to calculate parameter estimates and their associated unconditional standard errors (SE) based on QAICc weights (*wi ;* Burnham and Anderson 2002). In April 2014 and 2015, between 14 and 15 females of different sizes (range: 51-69 mm) from each island were kept in individual terraria until lying. Substrate, food, light conditions and basking sites for ovoposition were as in (Castilla, Bauwens & Price 2000). Eggs were counted, weighed and measured; the number of total eggs per female was used as proxy of fertility. Analyses were conducted using R 3.1.0 (R Development Core Team, 2008).

Results

Ecological characteristics of the islets

We have found a total of 124 species of vascular plants (Appendix A1). As expected, the number of species was positively associated with island size with 108 species at MO, 76 at NG and 15 at ES. Islets had floristic affinity because they are under the same climatic conditions and the influence of marine environment (Table 1). We recognized a zoning of four major vegetation types: coastal and nitro-halophytic communities (*Limonium spp.*, *Frankenia hirsuta*, *Arthrocnemum macrostachyum*, *Suaeda vera*), dune communities (*Lotus cytisoides*, *Helichrysum stoechas*, *Cakile maritima*), island shrub communities (*Pistacia lentiscus*, *Phillyrea latifolia subsp. media*), and herbaceous communities with a ruderal character (*Lavatera arborea*, *Chenopodium murale*, *Fumaria bastardii*). On the smallest islet (ES) only the halophytic community was present. Whereas in NG and MO it was possible to identify a zoning from the coastline (dune community) to the inland (shrub community; Fig 1). Biotic capacity index (D) was higher in MO and lower in ES (Table 1), as it was the proportion of annual flowering (Table 1).

Genetic analysis

Genetic structure and gene flow: The 15 microsatellites loci contained between 8 and 19 alleles, with a mean of 12.13 alleles per locus. Estimates using ENA correction method were very similar, indicating no presence of null alleles, scoring errors or linkage disequilibrium (see allele frequencies in appendix A2). Despite populations are closed; genetic diversity was high in all three populations and associated with island size. Relatedness values (r) corroborated these results with the highest level of inbreeding for ES population (r=0.083), but overall inbreeding level was small (Table 2). Unexpectedly, the islet with intermediate size (NG) had the lowest number of private alleles. Pairwise comparisons of genetic differentiation

showed significant differentiation between islands ($p<0.001$). F_{ST} and R_{ST} suggested that MO and NG were closely related while ES differed from the other two islets (Table 3). AMOVA analysis within population showed that 6% of variation was among populations and 94% within them ($p = 0.001$). Bayesian clustering showed two clusters ($K=2$) as the most likely number of genetic clusters, with MO and NG clearly assigned in the same cluster, and ES in a separate one (Fig 2). These results were consistent with the estimates of F_{st}, showing lower genetic structure between the closest islands (MO-NG). Accordingly, the PCA of the allelic frequencies of microsatellite loci revealed high levels of structure across islands with component 1 differentiating ES, and component 2 separating MO from NG (Fig 3). BayesAss analyses indicated a high proportion of genotypes consistent with their own population (approximately 94%, 97% and 98% in MO, NG and ES respectively). There was practically no recent gene flow among populations except between MO and NG, with about 4.7% of individuals in MO estimated to derived from NG ($m = 0.047$; 95% CI 0 - 0.103).

POP	N	A	A_E	H_O	H_E	A_P	r
ES	28	5.87±0.641	3.447±0.386	0.622±0.073	0.629±0.057	1.27±0.3	0.083
MO	27	9.73±0.643	5.618±0.396	0.815±0.025	0.806±0.017	2.6±0.466	0.015
NG	25	8.39±0.566	4.569±0.260	0.787±0.035	0.77±0.015	0.87±0.192	0.039

Table 2. Genetic diversity within islets. N, samples sizes; A±SE, average number of alleles; A_E±SE, effective number of alleles; H_O±SE, observed heterozygosity; H_E±SE, expected heterozygosity; A_P±SE, number of private alleles; r, relatedness.

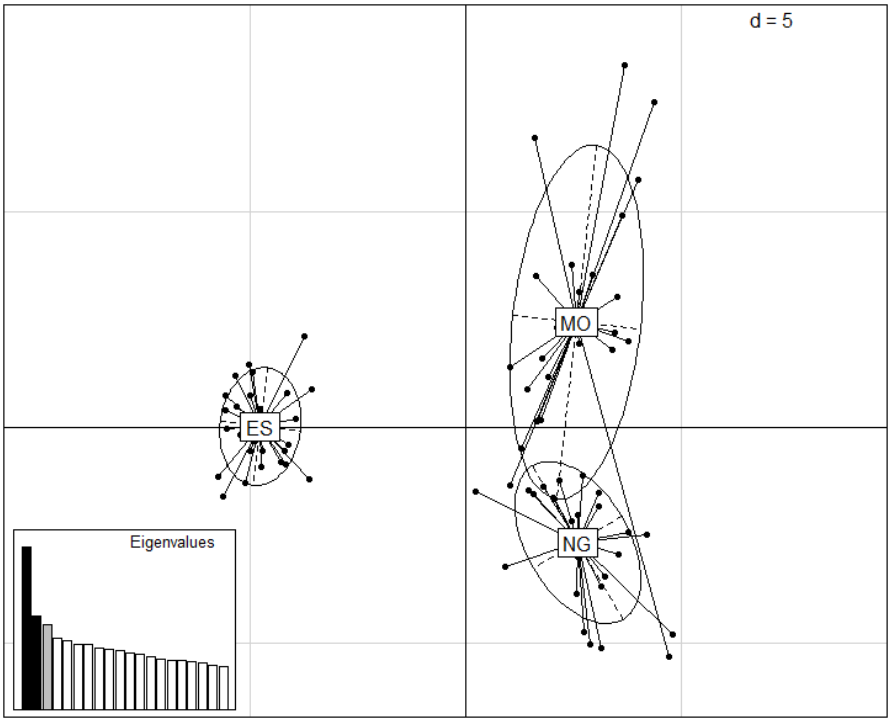

Fig. 2. First and second components of a principal components analysis (PCA) of 15-locus microsatellite genotypes from 80 insular lizards. Ovals are 95% inertia ellipses.

	ES-MO	ES-NG	MO-NG
F_{st}	0.135 (0.001)	0.144 (0.001)	0.03 (0.001)
R_{st}	0.025 (0.037)	0.024 (0.034)	0.044 (0.005)

Table 3. Pairwise comparisons of F_{ST} (Weir and Cockerham, 1984), and R_{ST} (Slatkin, 1995) among balearic lizard populations. *p* values are in brackets.

Demographic bottlenecks and population history (ABC analysis): All three populations showed lower values of M index M_{MO} = 0.57, M_{NG} = 0.56 and M_{ES} = 0.46, which are below M_{crit} of 0.68 (Garza and Williamson 2001). This

suggests the occurrence of a reduction of population size in the past. Excess heterozygosity was detected in the three populations only under the IAM mutation model, TPM mutation model was near significant in NG, an indication of a possible more recent bottleneck in NG (Table 4). This was in agreement with ABC results. Indeed, the most highly supported scenario was Scenario 3 that assumed NG population size diminished when human arrived and inhabited the island around 400 years. Posterior probability value (0.68) was similar to other published posterior probabilities for winning scenarios (Ghirotto *et al.* 2010; Lander *et al.* 2011). Type I error, i.e. the probability to reject a true scenario, for Scenario 3 was 0.18 (Table 5), while type II error rate, i.e. the probability to select a wrong scenario was between 0.06-0.1. Scenario 2, however, showed similar Type I and Type II error rates than Scenario 3. However, in our case the selection of Scenario 3 reached a high value (0.70) and the estimated duration of the predicted bottleneck (db) ranged between 40 and 76 generations (about 160-380 years).

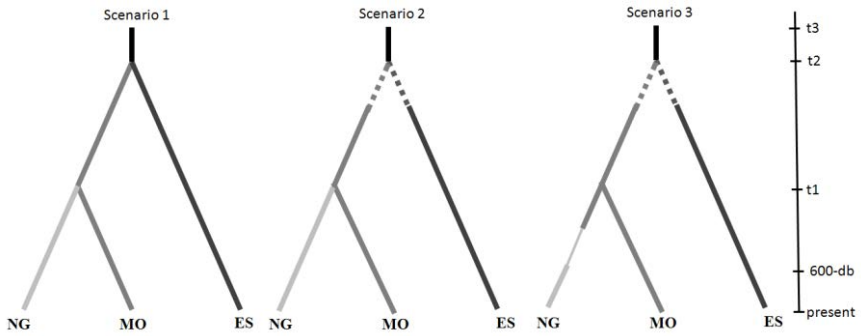

Fig. 3. Coalescent trees used in DIYABC analysis. Three scenarios assume 3 populations (MO, NG, ES) at present time. These population diverged from a single popularion. Dashed lines in branches indicate the possibility of population size change. Change in pattern size of the branches indicates a bottleneck (db: bottleneck duration). The time scale is on the right.

Demographic traits

As expected individuals at NG were smaller than those in the other two islets, but this difference was more pronounced in males (c. 8% smaller than in the MO and ES; Appendix A4).

Individual growth analysis: Growth parameters varied over sex and islet (see Appendix A5 for model selection). The statistical interaction between sex and population was dropped off for both, the characteristic growth rate (*K*) and the asymptotic size (*y2*). Model selection indicated that males had a significantly larger asymptotic size and characteristic growth rate than females. Moreover, we found statistically significant differences among islets. *K* parameter was smallest in ES while it was similar between MO and NG (MO: $y_{2♂}$= 74.86 ± 0.21; $K_{♂}$= 0.0023 ± 0.00007; $y_{2♀}$= 67.75 ± 0.20; $K_{♀}$= 0.0020 ± 0.00008. NG: $y_{2♂}$=68.46±0.46; $K_{♂}$= 0.0022±0.00022; $y_{2♀}$=61.35±0.52; $K_{♀}$=0.0019±0.00022. ES: $y_{2♂}$=73.02±0.42; $K_{♂}$=0.00172±0.00015; $y_{2♀}$=65.92±0.35; $K_{♀}$=0.00136±0.00015.).

Survival and fecundity analyses: The GOF test indicated the presence of transient animals in males of MO and NG, and in both sexes of ES (Table A1, Appendix A6). To correct for this, we eliminated the first observation from these datasets. Recapture probability was varying as a function of year and sex, with males more likely to be re-captured than females (see Table A3, Appendix A6). Model information theory suggested that survival can be assumed to be constant (Fig. 4). Generally males had a higher survival of females, but in ES. Average estimates indicated that survival was lowest in female at NG and higher in females at ES. We obtained a total of 105 eggs (MO: n= 49, NG: n = 27, ES: n = 29) from 42 females (MO: n =15, NG: n = 13, ES: n = 14). Females of MO laid more eggs than in ES and NG. One-way ANOVA revealed there was significant differences in number of eggs laid among islands ($F_{3,36}$ = 3.27, p = 0.049) being NG the population where females laid less number of eggs (Fig. 4). The

average number of eggs by population was 3.45±1.5 in MO, 2.23±1.01 in NG, and 2.60±1.03 in ES.

Population	Mutation Model	Heterozygote excess
MO (n = 27)	IAM	**$p = 0.001$**
	TPM	$p = 0.755$
	SMM	$p = 0.958$
NG (n = 25)	IAM	**$p < 0.0001$**
	TPM	**$p = 0.058$***
	SMM	$p = 0.820$
ES (n = 28)	IAM	**$p = 0.002$**
	TPM	$p = 0.380$
	SMM	$p = 0.768$

Table 4. Summary of the parameters and results for the BOTTLENECK program analyses used to detect significant recent reductions in effective population sizes. Significant *p* values in bold.

Discussion

Island populations of lizards show a remarkable degree of geographic differentiation in their morphological and demographic traits (Pérez-Mellado *et al*. 2008). In neighboring populations, this difference is generally ascribed to the isolated character and to the biotic characteristics of the islands. The role of past ecological environment can also be important, but it can only be studied indirectly by excluding other potential causes. Using ecological data, capture-recapture information and genetic analysis of three neighboring and isolated populations we have investigated the influence of multiple potential factors that may be

responsible for the morphological differentiation in adult body size in the Lilford's lizards. Genetic data indicated two main clusters, ES and MO+NG and migration analyses supported the isolated nature of the populations, with the exception of a small flow between NG and MO probably due to an occasional and recent translocation facilitated by humans visiting the islets. Despite NG showed a substantial degree of overlap with MO, its genetic diversity was lower and there has been a loss of private alleles (Frankham 1996). The low value of the Garza-Williamson (M value) index in the three populations suggested a size reduction in the past likely due to the rise of sea level occurred more than a thousand generations in the past (4-5000 years in the past; Berg 2015) probably followed by a process of genetic drift. The analysis of possible historical scenarios supported a change in population size, especially in ES, following the first isolation event between ES and MO+NG since the ancient population formed by MO+NG would be large enough to avoid genetic drift. The posterior isolation between MO and NG may explain why these two populations are so similar genetically and the low M ratio in the three populations. More interesting bottleneck analysis indicated an impact of a posterior and long-term human settlement in NG. Walsh (2006) showed that genetics-based changes in life history traits in populations that suffered constant human pressure loose the capacity to recover. NG is the population with the smallest lizards in the genetic cluster that it belongs along with MO, however there are more than 7 mm of difference in size between the two populations (Salvador 1980, 1986; Pérez-Mellado & Salvador 1988). Differences in size may be attribute to resources availability of each islet because lizards are very sensitive and their size may be constrained by a lack of resources (Dunham 1978; Wikelski, Carrillo & Trillmich 1997). However, the smallest islet (ES) were clearly different and characterised by a poorer floristic composition and a denser population size compared with the other two populations considered. Accordingly, individual-based data showed that lizards in this islet had a lower characteristic growth rate (*K*), and a higher annual

survival probability as expected in food limited populations (Andrews 1976; Le Galliard, Ferriere & Clobert 2005).

Type II error for each scenario				
Scenarios	1	2	3	Type I error
1	-	0.02	0.01	0.10
2	0.04	-	0.16	0.11
3	0.06	0.10	-	0.17

Table 5. Type I and Type II error when each of the scenarios was used to simulate data and DIYABC was used to select the most likely scenario for the simulated data.

We found an important difference in survival probability across the islets. The low survival of females in NG contrasted with the female survival in the other two islets. However, males showed slight trend to reduce survival with size due to a higher competition and territory defence (Sinervo *et al.* 2000; Shine 2005). A plausible explanation of this higher level of extrinsic mortality in NG could be a cost of early reproduction (Shine 1980). In the fertility study, it was seen that NG were the population with lesser number of eggs per females. Moreover, females from NG are smaller and may be the number of eggs laid was a physical question, however females form ES are larger and laid approximately the same number of eggs in average. Fecundity in reptiles depends on body size (Shine & Schwarzkopf 1992) and the energy allocation to reproduction at small sizes may result in additional cost to survive. Compared to MO and ES, NG seemed to adopt another type of strategy and closely follows the pattern observed in the mainland population or populations under predatory or hunting pressure (Hutchings 2004; Proaktor *et al.* 2007).

It is noted that the presence of a past selective force acting against larger sizes could select for early breeding individuals. Pregill (1986) suggested that human colonization of small islands have led to a significant

reduction in lizard body size caused by the human-induced loss of native habitat and the introduction of not-native predators, this fact may explain the smaller size of males in NG. Our data supported this scenario. DIYABC analysis suggested an important reduction in the effective population size in NG dating back about 620 generations (c. 2200 years before present) with a duration of bottleenck (db) about 60 generations. With a time of generation about 4-5 years, these estimates is in agreement the documented human settlement in NG, dated to Punic era (about 400 BC; Guerrero 2000). Remains of ceramic and iron objects in NG testified its use as a trading post for about 300-400 years. Animal bones found during excavations in the NG settlement indicate the presence of goats on the island during the Punic settlement (Guerrero 1997, p. 559) and changes in the original flora are likely to have occurred due to the grazing activity (see also (Pafilis *et al*. 2013). Rests of one carnivore, possibly a dog, are also documented for NG (Guerrero 1997, p. 559). The influence of past human-related selective pressures seemed the most likely explanation for the change in body size, which probably resulted in a new set of life history traits compared to the other two islets.

Our results stress the importance of considering the ancient human colonization as an additional evolutionary force that has shaped the present island fauna (Corti *et al*. 1999). Furthermore, these results could be comparable to the effects that now are suffering in overhunting or overfishing populations (reduction of size, early maturation and high mortality of larger sizes). Actually, these adaptative changes are showed irreversible or very long-lasting in our results, we emphasize the need to really take account these changes that are shaping current populations due to human pressure because there may be no return to recovery of the original population.

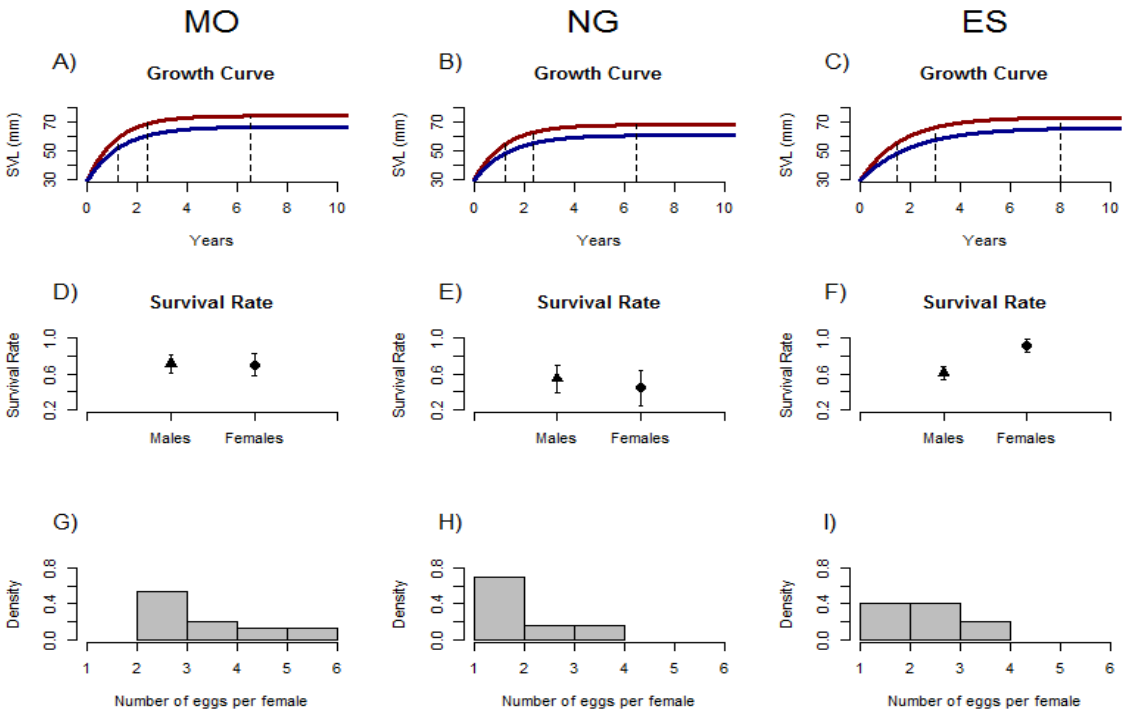

Figure 4. Demographic differences among the three populations. Individual growth rate of males (red line) and females (blue line) in MO, NG and ES (A,B and C) Dashed lines represent different stage (Juvenile, Sub-adult, Adult and Senior). Survival rate between males (red line) and females (blue line) of MO, NG and ES (D, E and F) in each size-state. The best fitted model (black lines) with CI (dashed lines) of the number of total eggs per female in a reproductive season in MO, NG and ES (G, H and I).

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Supplementary information

Appendix A1

List of plant species in each islet

	MOLTONA (MO)	NA GUARDIA (NG)	ES CURT (ES)
Phylum TRACHEOPHYTA			
Class PINOPSIDA			
CUPRESSACEAE			
Juniperus phoenicea *L. subsp.* ***turbinata*** *(Guss.) Nyman*	1	-	-
PINACEAE			
Pinus halepensis *Mill.*	1	1	-
Class GNETOPSIDA			
EPHEDRACEAE			
Ephedra fragilis *Desf. subsp.* ***fragilis***	1	-	-
Class MAGNOLIOPSIDA			
AIZOACEAE			
Mesembryanthemum nodiflorum *L.*	1	1	1
AMARANTHACEAE			
Arthrocnemum macrostachyum *(Moric.) Moris in Moris & Delponte*	1	1	1
Atriplex halimus *L.*	1	1	-
A. prostrata *Boucher ex DC. in Lam. & DC*	1	-	-
Beta vulgaris *L. subsp.* ***maritima*** *(L.) Arcangeli (Beta maritima L.)*	1	-	-
Chenopodium murale *L.*	1	1	-
Suaeda spicata *(Willd.) Moq.*	-	-	-
S. vera *Forssk. ex J.F.Gmelin*	1	1	1
ANACARDIACEAE			
Pistacea lentiscus *L.*	1	1	-

APIACEAE			
Bupleurum semicompositum L.	1	1	-
Crithmum maritimum L.	1	1	1
Eryngium maritimum L.	1	-	-
ASTERACEAE			
Aetheorhiza bulbosa (L.) Cass. subsp. *bulbosa*	1	1	-
Anthemis cf. arvensis L.	1	-	-
Asteriscus aquaticus (L.) Less.	1	-	-
Bellis annua L. subsp. *annua*	1	-	-
Calendula arvensis (Vaill.) L.	-	1	-
Carduus tenuiflorus Curtis	1	1	-
Filago pyramidata L. subsp. *pyramidata*	1	-	-
Glebionis coronaria (L.) Cass. ex Spach (= Chrysanthemum coronarium L.)	1	-	-
Hedypnois rhagadioloides (L.) F.W.Schmidt	1	-	-
Helichrysum stoechas (L.) Moench	1	1	-
Limbarda crithmoides (L.) Dumort. (Inula crithmoides L.)	-	1	-
Phagnalon saxatile (L.) Cass.	1	1	-
Reichardia tingitana (L.) Roth.	1	1	-
Senecio vulgaris L.	1	-	-
Sonchus asper (L.) Hill subsp. *asper*	1	1	-
S. oleraceus L.	1	1	-
S. tenerrimus L.	-	1	-
Urospermum picroides (L.) Scop. ex F.W.Schmidt	-	1	-
BORAGINACEAE			
Echium parviflorum Moench	-	1	-
E. sabulicola Pomel subsp. *decipiens* (Pomel) G.Klotz	1	1	-
Heliotropium europaeum L.	1	-	1
BRASSICACEAE			
Cakile maritima Scop. subsp. *maritima*	1	1	-
Diplotaxis ibicensis (Pau) Gómez Campo	1	1	-
Hornungia procumbens (L.)	-	1	-
Lobularia maritima (L.) Desv. subsp. *maritima*	-	1	-
CAMPANULACEAE			

Campanula erinus L.	1	-	-
CARYOPHYLLACEAE			
Arenaria leptoclados (Rchenb.) Guss.	1	-	-
Cerastium glomeratum Thuill.	1	-	-
C. pumilum Curtis	1	-	-
Herniaria cinerea DC. in Lam. & DC.	1	-	-
Polycarpon tetraphyllum (L.) L. subsp. *tetraphyllum*	1	-	-
Sagina apetala Ard.	1	-	-
S. maritima G.Don	1	1	-
Silene decipiens Barceló	1	-	-
S. sclerocarpa Léon Dufour	1	1	-
Spergularia bocconei (Scheele) Graebner in Asch. & Graebner	1	1	-
S. marina (L.) Besser	1	-	1
Stellaria pallida (Dumort.) Piré	1	-	-
CRASSULACEAE			
Crassula tillaea Lest.-Garl.	1	-	-
EUPHORBIACEAE			
Euphorbia peplus L.	1	1	-
E. terracina L.	1	1	-
Mercurialis ambigua L. fil.	1	1	-
FABACEAE			
Lotus cytisoides L.	1	1	1
L. edulis L.	1	-	-
Medicago littoralis Rohde ex Loisel.	1	1	-
Ononis reclinata L.	1	-	-
FRANKENIACEAE			
Frankenia hirsuta L.	1	1	1
F. pulverulenta L.	1	1	1
GENTIANACEAE			
Blackstonia acuminata (W.D.J. Koch & Ziz) Domin	1	-	-
Centaurium tenuiflorum (Hoffmanns & Link) Fritsch	1	1	-
GERANIACEAE			
Erodium chium (L.) Willd.	1	1	-

E. malacoides (L.) L'Hér. in Aiton subsp. *malacoides*	1	-	-
Geranium molle L.	1	-	-
G. purpureum Vill.	1	-	-
G. rotundifolium L.	1	-	-
LAMIACEAE			
Teucrium dunense Sennen	1	1	-
LINACEAE			
Linum strictum L.	1	1	-
MALVACEAE			
Lavatera arborea L.	1	1	-
L. cretica L.	1	1	-
OLEACEAE			
Olea europaea L. subsp. *europaea* L. var. *sylvestris* Brot.	1	1	-
Phillyrea latifolia L. subsp. *media* (L.) P.Fourn.	1	1	-
PAPAVERACEAE			
Fumaria bastardii Boreau	1	1	-
F. capreolata L.	1	-	-
Glaucium flavum Crantz	-	1	-
PLANTAGINACEAE			
Plantago afra L.	1	-	-
Pl. coronopus L.	1	1	-
Pl. lagopus L.	1	1	-
PLUMBAGINACEAE			
Limonium caprariense (Font Quer & Marcos) Pignatti	1	1	1
L. companyonis (Gren. & Billot) Kuntze	1	-	-
L. echioides (L.) Miller	1	1	-
L. virgatum (Willd.) Fourr.	1	-	-
POLYGONACEAE			
Rumex bucephalophorus L. subsp. *gallicus* (Steinh.) Rech. fil.	1	1	-
PRIMULACEAE			
Anagallis arvensis L.	1	1	-
RANUNCULACEAE			
Clematis cirrhosa L.	1	-	-

RUBIACEAE			
Galium murale (L.) All.	1	-	-
Rubia peregrina L.	1	1	-
Theligonum cynocrambe L.,	1	-	-
Valantia muralis L.	-	1	-
SANTALACEAE			
Osyris alba L.	1	-	-
URTICACEAE			
Urtica membranacea Poiret in Lam.	1	1	-
Class LILIOPSIDA			
AMARYLLIDACEAE			
Allium ampeloprasum L.	1	-	-
A. roseum L.	-	1	-
A. subvillosum Salzm. ex Schult. & Schult. fil. in Roem. & Schult.	1	1	-
Pancratium maritimum L.	1	1	-
ARACEAE			
Arisarum vulgare Targ.-Tozz.	1	1	-
Arum pictum L. subsp. *sagittifolium* Rosselló & L.Sáez	1	-	-
ASPARAGACEAE			
Asparagus horridus L.	1	1	1
Drimia maritima (L.) Stearn (Urginea maritima (L.) Baker)	1	1	-
Leopoldia comosa (L.) Parl. (Muscari comosum (L.) Mill.)	1	1	-
IRIDACEAE			
Gladiolus communis L.	1	1	-
Romulea columnae Sebast. & Mauri	1	-	-
JUNCACEAE			
Juncus acutus L. subsp. *acutus*	-	1	-
ORCHIDACEAE			
Ophrys L. sp.	1	-	-
POACEAE			
Avena barbata Pott ex Link	1	1	-
Bromus rigidus Roth	1	1	-
Dactylis glomerata L.	1	-	-

Catapodium marinum (L.) C.E.Hubb. (*Desmazeria marina* (L.) Druce)	1	1	1
C. rigidum (L.) C.E.Hubb. subsp. *rigidum* (*D. rigida* (L.)	1	1	-
Gastridium ventricosum (Gouan) Schinz & Thell.	-	1	-
Hordeum murinum L. subsp. *leporinum* (Link) Arcangeli	1	1	-
Rostraria cristata (L.)	1	-	-
Lagurus ovatus L.	1	1	-
Lolium rigidum Gaudin	1	-	-
Parapholis incurva (L.) C.E.Hubbard	1	1	1
Oryzopsis miliacea (L.) Asch. & Schweinf. subsp. *miliaceum*	1	1	-
Polypogon maritimus Willd.	-	1	-
Sporobolus pungens (Schreb.) Kunth	-	-	1
Triplachne nitens (Guss.) Link	1	1	1
Vulpia fasciculata (Forssk.) Samp.	-	1	-
	108	76	15

Appendix A2

Allele frequencies in the three populations of lizards.

Locus	Allele/n	ES	MO	NG
Pli2				
	228	0.260	0.019	0.000
	236	0.060	0.000	0.000
	240	0.160	0.019	0.000
	244	0.000	0.093	0.021
	248	0.000	0.167	0.146
	252	0.060	0.000	0.083
	256	0.160	0.148	0.125
	258	0.040	0.000	0.000
	260	0.000	0.241	0.313
	264	0.000	0.056	0.104
	266	0.000	0.019	0.000
	268	0.220	0.130	0.042

	272	0.020	0.037	0.104
	276	0.000	0.074	0.063
	284	0.020	0.000	0.000
Pli3				
	225	0.021	0.000	0.000
	235	0.292	0.000	0.109
	239	0.000	0.000	0.043
	241	0.000	0.058	0.022
	243	0.042	0.058	0.043
	247	0.375	0.192	0.326
	251	0.042	0.115	0.239
	255	0.063	0.173	0.065
	259	0.146	0.327	0.152
	263	0.021	0.019	0.000
	267	0.000	0.058	0.000
Pli4				
	360	0.130	0.058	0.045
	364	0.000	0.038	0.000
	366	0.000	0.019	0.000
	370	0.043	0.077	0.250
	372	0.109	0.135	0.068
	374	0.022	0.000	0.045
	376	0.435	0.288	0.386
	380	0.261	0.327	0.159
	388	0.000	0.019	0.000
	400	0.000	0.038	0.000
	404	0.000	0.000	0.045
Pli5				
	364	0.020	0.080	0.022
	366	0.200	0.120	0.196
	370	0.100	0.040	0.065
	372	0.000	0.020	0.000
	376	0.120	0.060	0.152
	380	0.000	0.180	0.000
	384	0.000	0.040	0.000

	388	0.320	0.380	0.326
	390	0.000	0.020	0.000
	392	0.020	0.020	0.022
	394	0.220	0.000	0.217
	398	0.000	0.040	0.000
Pli6				
	370	0.000	0.104	0.000
	374	0.024	0.021	0.304
	378	0.452	0.229	0.543
	380	0.000	0.021	0.000
	382	0.000	0.104	0.000
	386	0.000	0.021	0.000
	390	0.024	0.000	0.000
	396	0.238	0.125	0.000
	402	0.167	0.167	0.000
	404	0.000	0.021	0.000
	406	0.000	0.042	0.000
	410	0.095	0.021	0.022
	412	0.000	0.104	0.087
	416	0.000	0.021	0.043
Pli8				
	113	0.020	0.000	0.000
	131	0.000	0.000	0.091
	135	0.100	0.096	0.000
	139	0.040	0.135	0.386
	143	0.380	0.212	0.091
	147	0.140	0.288	0.250
	149	0.020	0.000	0.000
	151	0.000	0.115	0.136
	155	0.260	0.038	0.000
	159	0.040	0.000	0.000
	163	0.000	0.019	0.000
	167	0.000	0.038	0.000
	169	0.000	0.038	0.000
	173	0.000	0.000	0.045

	179	0.000	0.019	0.000
Pli9				
	381	0.000	0.083	0.000
	385	0.000	0.063	0.136
	389	0.000	0.104	0.114
	393	1.000	0.583	0.364
	397	0.000	0.167	0.386
Pli10				
	235	0.019	0.000	0.000
	239	0.231	0.037	0.000
	247	0.000	0.074	0.000
	251	0.000	0.000	0.060
	252	0.019	0.000	0.000
	255	0.000	0.167	0.300
	257	0.000	0.167	0.060
	259	0.019	0.093	0.060
	261	0.000	0.167	0.040
	263	0.673	0.019	0.160
	265	0.000	0.167	0.220
	267	0.000	0.037	0.000
	269	0.038	0.037	0.000
	271	0.000	0.037	0.060
	279	0.000	0.000	0.040
Pli12				
	170	0.060	0.000	0.000
	178	0.080	0.000	0.000
	192	0.000	0.043	0.000
	200	0.200	0.065	0.020
	202	0.100	0.065	0.000
	204	0.020	0.000	0.000
	206	0.040	0.022	0.000
	210	0.260	0.087	0.000
	212	0.000	0.022	0.020
	214	0.180	0.087	0.000
	216	0.000	0.022	0.000

	218	0.000	0.043	0.160
	220	0.000	0.065	0.000
	224	0.060	0.348	0.200
	228	0.000	0.043	0.340
	232	0.000	0.022	0.000
	236	0.000	0.000	0.020
	238	0.000	0.043	0.220
	246	0.000	0.022	0.020
Pli16				
	428	0.288	0.000	0.000
	432	0.000	0.000	0.158
	444	0.000	0.250	0.263
	448	0.635	0.000	0.000
	452	0.077	0.115	0.053
	456	0.000	0.192	0.474
	460	0.000	0.231	0.000
	463	0.000	0.173	0.053
	466	0.000	0.038	0.000
Pli17				
	233	0.000	0.037	0.000
	235	0.089	0.259	0.220
	239	0.804	0.093	0.440
	243	0.107	0.130	0.060
	247	0.000	0.148	0.040
	251	0.000	0.074	0.020
	255	0.000	0.130	0.220
	259	0.000	0.093	0.000
	263	0.000	0.019	0.000
	265	0.000	0.019	0.000
Pli18				
	123	0.000	0.058	0.021
	125	0.093	0.154	0.042
	127	0.000	0.038	0.021
	129	0.148	0.000	0.021
	131	0.241	0.462	0.354

	135	0.481	0.000	0.104
	137	0.000	0.019	0.104
	139	0.037	0.058	0.063
	143	0.000	0.154	0.146
	147	0.000	0.038	0.042
	149	0.000	0.019	0.021
	151	0.000	0.000	0.063
Pli21				
	132	0.000	0.019	0.000
	137	0.143	0.038	0.000
	138	0.054	0.000	0.000
	142	0.000	0.135	0.000
	146	0.393	0.250	0.174
	150	0.125	0.019	0.065
	154	0.036	0.346	0.348
	158	0.196	0.019	0.152
	162	0.054	0.077	0.043
	164	0.000	0.019	0.000
	166	0.000	0.019	0.087
	168	0.000	0.000	0.065
	170	0.000	0.058	0.065
Pli22				
	103	0.000	0.083	0.065
	107	0.000	0.313	0.283
	113	0.635	0.000	0.000
	115	0.000	0.146	0.022
	117	0.000	0.021	0.000
	119	0.365	0.229	0.217
	123	0.000	0.021	0.065
	127	0.000	0.188	0.348
Pli24				
	104	0.000	0.000	0.167
	107	0.000	0.058	0.333
	108	0.000	0.192	0.143
	110	0.214	0.038	0.000

112	0.000	0.212	0.238
116	0.167	0.115	0.048
118	0.000	0.096	0.000
120	0.000	0.154	0.048
122	0.024	0.000	0.000
124	0.024	0.058	0.000
126	0.333	0.000	0.000
128	0.071	0.038	0.000
132	0.167	0.038	0.024

Appendix A3

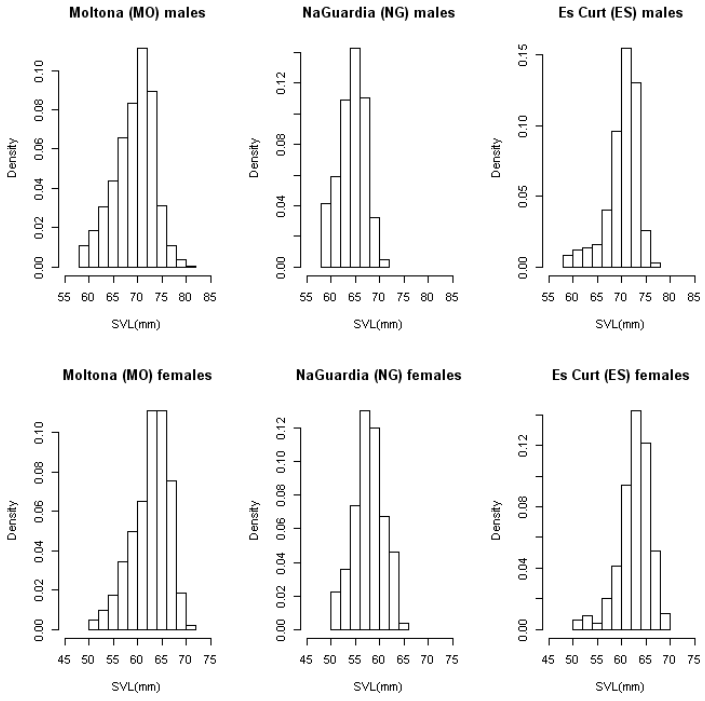
Population history (ABC analysis)

Coalescent models were compared by estimating their posterior probabilities using the logistic regression method (Ghirotto et al. 2010). Assuming a generation time of 4-5 years, prior values for time steps t1 and t2 were among 500–3000 generations (Berg, M.P. van den 2015). As prior values for population parameters we chosen the estimates of population size derived by Spatially Explicit Capture Recaptur models (SECR; Efford 2004) as in Ruiz de Infante Anton et al. (see also Rotger et al 2016; Appendix A3). The prior values for Scenario 3 force a population bottleneck 600 generations ago (around 2400 years). Population size change was allowed at each population divergence at t2. Prior values for effective population size at each time step and divergence time (t) were the following:

Time step	Population	Minimum range (N)	Maximum range (N)
t3 (ancestral)	MO+NG+ES	100	10000
t2	MO+NG	500	5000
t2	ES	50	500
t1	MO	100	3000
t1	NG	100	2000
t1	ES	50	500
0	MO	100	3000
0	NG	100	2000
0	ES	50	500

Appendix A4

Size frequencies between males and females in each islet.



Appendix A5

Modelling individual growth according to sex and islet. AIC = Akaike's Information Criterion. The best model are in bold.

MODEL	y_2	K	AIC
1	cte	cte	15928.72
2	cte	sex	15712.48
3	cte	island	15834.97
4	cte	sex+island	15908.09
5	cte	sex*island	15808.23
6	sex	cte	15753.23
7	sex	sex	15758.47
8	sex	island	14976.61
9	sex	sex+island	14976.00
10	sex	sex*island	14946.08
11	island	cte	15673.84
12	island	sex	15288.99
13	island	island	15181.62
14	island	sex+island	15284.82
15	island	sex*island	15285.51
16	sex+island	cte	14611.47
17	sex+island	sex	14602.02
18	sex+island	island	14577.22
19	**sex+island**	**sex+island**	**14559.64**
20	sex+island	sex*island	14578.75
21	sex*island	cte	14607.13
22	sex*island	sex	14598.81
23	sex*island	island	14575.51
24	sex*island	sex+island	14577.03
25	sex*island	sex*island	14580.28

Appendix A6

Table A1. Test of goodness of fit with the original data. Significant tests are in bold.

	MO		NG		ES	
	Females	*Males*	*Females*	*Males*	*Females*	*Males*
Ztransient	0.62098	**3.2174**	0.74852	1.8664	1.5355	-1.6312
Test 3Sr	3.5179	**15.5979**	1.1206	**9.7798**	**9.6852**	**11.6043**
Test 3Sm	1.8734	2.3129	0	0.32646	2.6648	0.40239
Ztrap	-0.8848	**-2.1869**	-0.97792	-0.67466	**-2.6506**	-0.73747
Test 2CL	1.11	5.5107	1.9127	0.69413	**9.5752**	1.6316
Test 2 CT	2.3381	0.70833	0	0.58478	1.042	0.8253
Total	8.8394	**24.12983**	3.0333	**11.38517**	**22.9672**	**14.46359**

Table A2. Test of goodness of fit removing the first observation in the datasets where the original GOF test was significant.

	MO		NG		ES	
	Females	*Males*	*Females*	*Males*	*Females*	*Males*
Test 3Sr	2.943	**13.11**	1.12	**8.507**	8.177	9.503
Test 3Sm	1.503	2.313	0	0.326	3.375	0.395
Test 2CL	1.386	5.317	1.913	0.439	7.967	1.397
Test 2 CT	5.83	19.4	3.033	9.27	19.5	11.3
Total	11.662	40.14	6.066	18.542	39.019	22.595
c-hat	1	1.62	1	1	1.95	1.13

Table A3. Model selection

Models	AICc	*k*	Dev	w_1
MO				

Φ(sex)p(time)	257.93	7	45.37	0.31
Φ(.)p(time)	258.20	6	47.80	0.27
Φ(sex)p(sex+time)	259.33	8	44.58	0.15
Φ(sex)p(sex*time)	261.00	10	41.80	0.07
Φ(.)p(sex*time)	261.38	10	42.19	0.05
Φ(time)p(sex)	261.42	7	48.86	0.05
NG				
Φ(.)p(time)	173.36	5	12.84	0.21
Φ(time)p(.)	175.05	5	14.52	0.09
Φ(sex)p(sex+time)	175.14	6	12.40	0.09
Φ(sex+time)p(sex+time)	175.25	8	7.96	0.08
Φ(.)p(sex*time)	175.26	8	7.97	0.08
Φ(sex)p(time)	175.31	6	12.57	0.08
Φ(time)p(time)	176.00	7	11.01	0.06
Φ(sex+time)p(sex)	176.07	6	13.34	0.05
ES				
Φ(sex)p(sex*time)	479.33	8	29.80	0.55
Φ(sex*time)p(sex*time)	481.63	11	25.68	0.17
Φ(sex*time)p(sex)	481.97	9	30.31	0.15
Φ(sex)p(sex+time)	483.03	7	35.62	0.09

Chapter IV

Contrasting size-dependent life history strategies in female lizards from insular close populations

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Journal of Animal Ecology (*under review*)

Contrasting size-dependent life history strategies in female lizards from insular close populations

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Summary

1 In many species with continuous growth, body size is an important driver of life-history tactics and its relative importance is thought to reflect the spatio-temporal variability of selective pressures.
2 We developed a size-dependent integral projection model (IPM) for three insular lizard populations with contrasting realized adult body sizes to investigate how size-related selective pressures can influence lizards' life-cycle.
3 For each population we investigated how body size influenced different demographic rates. We decomposed differences in population growth rates into contributions from size-dependent growth rate, survival, and fecundity.
4 Survival was generally unrelated to body size, except in the island with the smallest average body sizes where mortality increased with snout-to-vent length. Furthermore, in this population the number of eggs per female, which was used as a prior of fecundity, it was unrelated to female body size, while the association was positive in the other two populations.
5 Elasticity analyses revealed that a size-independent fertility was compensated for a size-dependent mortality. Indicating how size-dependent selective pressures can generate different life-history strategies in three close populations

Key-words: Body size, capture-recapture, integral projection model, Lilford's lizard, size-dependent mortality.

Resum

En moltes espècies amb creixement continu, la mida del cos és un motor important de les tàctiques d'història de vida i la seva importància relativa es considera que reflecteix la variabilitat espai-temporal de les pressions selectives.

Hem desenvolupat un model de projecció integral dependent de la mida (IPM) per a tres poblacions de sargantanes insulars que es diferencien per la mida que arriben a assolir els adults, per investigar com les pressions selectives relacionades amb la mida poden influir al cicle de vida de les sargantanes. Per cada població es va investigar la manera que influeixen en la mida del cos diferents paràmetres demogràfics. Es van descompondre les diferències en les taxes de creixement de població en les contribucions depenent de la mida que són la supervivència i la fecunditat.

La supervivència no va estar relacionada generalment amb la mida corporal, excepte a l'illa on la mida corporal mitjana és més petita, on la mortalitat va augmentar amb la mesura del cos. A més, en aquesta població el nombre d'ous per femella, que es va utilitzar com aproximació de la fecunditat, no estava relacionat amb la mida del cos de la femella, mentre que la relació va ser positiva en les altres dues poblacions.

Les anàlisis d'elasticitat van revelar que la independència de la mida a la fecunditat era compensada per una mortalitat depenent de la mida, indicant com les pressions selectives dependents de la mida poden generar diferents estratègies de vida en tres poblacions properes.

Paraules clau: Mida del cos, captura-recaptura, models de projecció integral, sargantana de Lilford, mortalitat depenent de la mida.

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Introduction

Individual body size is an important determinant of life-history tactics in many taxa (Peters 1986). Owing to its pivotal role in multiple evolutionary tradeoffs, the dynamics of body size within and between populations can only be studied considering the whole life-cycle of an organism (Roff 1992; Stearns 1992; Charnov 1993). Reptiles are good biological models to track the importance of body size in animal demography. Although the

growth rate abates throughout an individual lifespan, body growth is continuous (Schoener & Schoener 1978; Smith, Amarello & Goode 2010). In many reptile species, large individuals show social dominance over smaller ones generating asymmetric trophic and social interactions (Massot et al. 1992; Lecomte et al. 1994; Mugabo et al. 2010). However, realized body size at early age or stage is mainly determined by environmental conditions and might thus constraint optimal decisions and individual fitness (Rotger et al. 2016). For example, small breeding females might produce small eggs and have lower breeding or hatching success (Laurie & Brown 1990). Large sizes might also be constraint, for example, larger individuals may be more easily detected by predators than smaller ones (Stamps & Buechner 1985; Blanckenhorn 2000). Given this variability, it is plausible to assume that the relative role of body size is specific to each population. Here, we used the recently developed Integral Projection Models (IPMs; Easterling, Ellner & Dixon 2000) which allows the characterization of an organism' life-cycle as a function of a continuous trait (Easterling et al. 2000; Ellner & Rees 2006; Rees & Ellner 2009; Coulson, Tuljapurkar & Childs 2010; Coulson 2012), thereby providing a framework to address hypotheses on eco-evolutionary dynamics (Rees & Ellner 2009; Chung, Miller & Rudgers 2015) or population management (Wallace, Leslie & Coulson 2013). Beside the technical advantage, it has been shown that IPMs offer a more realistic model to investigate the role of body size in species with indeterminate growth (Bassar et al. 2013; Wallace et al. 2013). Therefore, we performed an IPM to investigate the role of body size in shaping life-history tactics in three isolated populations of Lilford's wall lizards (*Podarcis lilfordi* Günther 1874). The Lilford's lizard is an endemic species of the Balearic archipelago (Spain). The species disappeared from the main islands of Mallorca and Menorca and it is now confined to offshore islets and to the islands of Cabrera (Alcover, Seguí & Bover 1999). Terrasa et al. (2009) described four main genetic clades, but this genetic structure does not match the distribution of body size across suggesting that this trait

evolved independently of genetic relationships. We focused our investigations on three geographically close populations belonging to the same genetic clade, i.e. clade III in Terrassa et al. (2009). The three populations show a marked difference in the adult average SVL (Pérez-Mellado et al. 2008). In each population the sources of variation were described by the association between individual snout-vent length (SVL), a standard measure of body size for reptiles, and four demographic functions, namely the survival probability, the body growth, the reproduction and inheritance (see Coulson et al. 2010). Since size variation can affect life-history traits and population-dynamics (Clutton-Brock et al. 1987; Rose, Clutton-Brock & Guinness 1998) we expect to find differences in the life-cycle and the realized population growth rate of the species depending on the population considered. If a small body size is maintained by phenotypic selection, we expect to find a cost of being large in the island with the smallest average SVL (Blanckenhorn 2000). This phenotypic disadvantage might be expressed by a negative relationship between SVL and body growth, survival and/or fecundity

Materials and methods

Study area and individual based-data

Males of the Lilford's lizard are approx. 10% larger than females, but body size and sexual size dimorphism can vary across populations (Salvador 1980, 1986). Lizards peak of births occurring around June but the mating period goes from early March to the end of September (Pérez-Mellado & Salvador 1988; Castilla, Bauwens & Price 2000). Newborn lizards measure about 30mm long and laboratory data suggest that females begin to breed between they first and second year, at a SVL of 50mm (Castilla & Bauwens 2000). The species reduces its activity during the winter but does not hibernate (Salvador 1986; Rotger *et al.* 2016). To avoid confounding effects of climate or genetic structure, we focussed on three neighbouring islets (average distance: 2.2Km) from the same genetic clade: Na Moltona

(5.09 ha, MO hereafter), Na Guardis (1.98 ha, NG hereafter) and Es Curt (0.29 ha, ES). Despite these similarities, animals in NG are about 10-14% smaller than those in other islands. In each islet, lizards were captured in pitfall traps during three-day capture-recapture session in October and April (Ruiz de Infante *et al*. 2014). For MO data were collected from 2009 to 2015 while for NG and ES data collection began in 2010. Each captured lizard was sexed, weighed, and measured from snout to vent (SVL) to the nearest mm and photographed using a digital camera for individual identification (Sacchi *et al*. 2010; Moya *et al*. 2015). Individuals were released near the trap of capture to maintain the spatial structure of the population.

Size-dependent survival analysis

Size-dependent annual survival of females was estimated from October to October through multistate capture-recapture models for stratified data (Schwarz *et al*. 1993). Multistate capture-recapture models estimate the state-dependent survival and between states transition together with state-dependent probability of recapture. However, time-dependent individual covariates, such as weight or body size, cannot be easily accommodated because covariate values are unknown when an individual is not caught. To estimate size-dependent annual survival we stratified observations in 5 state-classes identified by combining known aspects of *P. lilfordi* ecology with the length-by-age growth curve estimate for each population using all data available (see Supplementary data). The five state-classes considered were: individuals born in the previous spring ('newborns', hereafter, between 3.2-4.2 mm), 'juvenile' females of c. 1 year old but under the size of maturation (4.2-5.2 mm; (Castilla *et al*. 2000), individuals in their first reproductive season ('subadults', hereafter, 5.2-6.0 mm), adult females between the size of first reproduction and the maximum asymptotic size estimated by the growth curve ('adults', 6.0-6.4mm) and females that have reached or exceeded the maximum

asymptotic size ('seniors',. >6.4mm). The probability to move from a size-class *r* to a class *s*, ^{rs}, approximates the growth rate. We built a vector including the observed average body size in size-class *j*, B_j with j=1,2, … 5, and constrained state-dependent survival estimates to be a linear function of the observed size values as:

$$\text{Logit}(S^r) = \alpha + \beta Bj$$

where and are the linear predictors of the size-dependent survival (see a similar approach in Fernández-Chacón *et al*. 2015). Models were built in program MARK (White & Burnham 1999) and goodness of fit test was assessed with program U_CARE (Choquet *et al*. 2009).

Annual growth rate

Annual observed growth from SVL *x* at year *t* to SVL size *y* at year *t*+1 was estimated using individuals recaptured during the October sessions only as :

$$E(y) = a_g + b_g x$$

Finally, we estimated the conditional variance, var(*y*), using the squared residuals of the regression on size at time *t*.

	SURVIVAL						GROWTH			TOTAL EGGS		
Islet	n	Newborn	NonBreeder	Subadult	Adult	Mature	n	SVL*t* (mm)	SVL*t+1* (mm)	n	eggs/female	SVL females
MO	324			0.61 (0.04)			129	61.33 (4.57)	65.30 (2.58)	49	3.50 (1.56)	65.28 (2.46)
NG	197	0.99 (0.02)	0.89 (0.10)	0.70 (0.10)	0.51 (0.10)	0.33 (0.14)	40	57.07 (3.65)	59.97 (2.25)	27	2.25 (1.05)	59.25 (3.39)
ES	246			0.79 (0.03)			135	61.55 (3.56)	63.51 (2.65)	29	2.42 (1.16)	64.50 (3.63)

Table 1. Number, survival of each class, mean snout-vent length (SVL) at time t (SVL1) and t+1 (SVL2) of females of each population, and number of total eggs with the mean number of eggs laid per female and their mean SVL. Values in parentheses are standard errors.

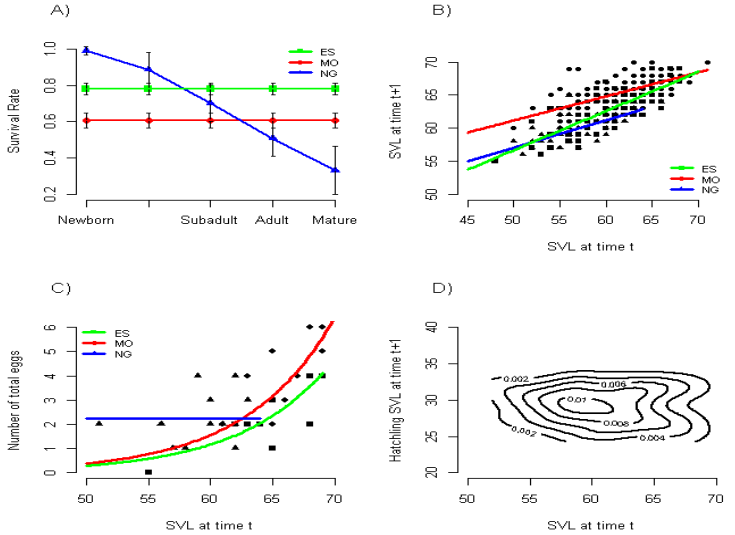

Fig. 2. Stable size distribution (black line) and reproductive value (dashed line) feom the integral projection model in three populations (see text for details).

Size-dependent fertility

The association between fertility and body size was estimated using experimental data. In April 2014 and 2015, 5 to 8 females from each island were kept in individual terraria until lying. Substrate, food, light conditions and basking sites for ovoposition were as in Castilla & Bauwens (2000). In 2015, 3 to 5 males for each island were left with the respective females to ensure fertilization. All females were released in August in the same site of capture. Eggs were weighed and measured and incubated at a stable temperature (27-29 °C) and humidity (80%-90%) in an egg-incubator. In 2015, when males were present, eight eggs hatched. Given the limited number of hatchlings, we used the number of eggs as proxy of fecundity. We used Poisson's regressions to estimate the relationship between female SVL and the number of eggs laid in program R (R Development Core Team 2011).

Integral population Model and elasticity analyses

We parameterized a deterministic, post-breeding, IPM based on the estimated relationship between vital rate and SVL. As above, we note SVL at year *t* as 'x' and SVL at year *t*+1 as "y" in the formulae. We refer to the size domain of *x* as Ω. :

$$n(y, t+1) = \int_{\Omega} P(y,x) + F(y,x)n(x,t)dx$$

Where the function P(y, x), represents the size-dependent survival for individuals of size *x* as:

$$P(y,x) = s(x)G(y,x)$$

where, G(y,x) is the probability of growing from size *x* to size *y* in one year. Similarly, F(y, x) represents the production of *y*-sized offspring from *x*-sized parents at time *t*. The fertility function is represented by:

$$F(y,x) = r(x)p_{HS}D(y,x)$$

being r(x) the recruitment function describes the number of total eggs produced by a x-sized female and D(y, x) the inheritance function describes the probability that a reproducing female of size *x* at year *t* produces a recruit of size *y* at year *t* + 1. These two functions are multiplied by p_{HS}, the size-independent probability of hatching. These demographic functions together constitute the kernel of the IPM, which describes all possible transitions between sizes in a single time-step, e.g. year (Rees, Childs & Ellner 2014). The function is multiplied by 0.5 to account for female only (note that in this species the gender is genetically determined therefore we assume equal sex ratio). Our final kernel equation is:

$$K(y,x) = s(x)G(y,x) + s(x)r(x)p_{HS}D(y,x)/2$$

The IPM projection kernel for each population was discretized into a matrix with 300 size classes and analyzed using commonly used techniques in matrix population models (Easterling *et al.* 2000; Ellner & Rees 2006). IPMs were built in R 3.2 (R Development Core Team 2011). Elasticities are used to measure the proportional change in the population growth rate, λ, caused by proportional changes in $P(y,x)$ and $F(y,x)$ (Tuljapurkar 1990). We computed the elasticities of λ in each islet and partitioned into contributions from survival-growth and fecundity components of the kernel (Ellner & Rees 2006).

Results

Size-dependent survival

Survival probability was estimated using a total of 767 females (MO = 324, NG = 197, ES = 246) between 2009 and 2015 during the October sessions only. The goodness-of-fit suggested that the general model fit the data adequately in all populations (MO: χ^2 = 17.70, df = 36, p = 0.99; NG: χ^2 =

7.84, df = 14, p = 0.90; ES: χ^2 = 26.61, df = 39, p = 0.86; see Supplementary data for GOF details). Individual SVL influenced survival only in NG while in MO and ES a model assuming a size-independent survival was retained (Table 2; Fig 1A). The relationship between SVL and survival in NG was negative may indicate a strong senescence of female (Table 1).

Size-dependent growth rate analysis

We gathered data on 305 females (MO = 129, NG = 50, ES = 135) to estimate the growth function between Octobers ($G(y,x)$). Growth had a positive linear trend in the three populations (Table 2; Fig 1B). Growth rate was faster in MO compared with the other two islets (Table 2). Variation among years was no significant and it was not considered in the model (Table 2).

Size-dependent fertility

We obtained a total of 105 eggs (MO: n= 49, NG: n = 27, ES: n = 29) from 39 females (MO: n =14, NG: n = 12, ES: n = 12). We found a positive relationship between female size and number of eggs laid in MO and ES (Table 2. Fig 1C). In contrast, the number of eggs in NG (average: 2.25±1.05) was independent from female size. Mean SVL at hatchling was 29.3±2.5 mm (n = 15). Offspring size did not depend on mother size (t = 1.98, p = 0.08), but sample size was too low to assess a mother-offspring size-relationship in each islet. However, Castilla & Bawens (2000) using with a larger dataset from a population of the same genetic clade did not find any relationship between mother and offspring size. Consequently we assumed that the change of offspring's body size in relation to parents' SVL was unimportant (Table 1; Fig 1D, see also Wallace et al. 2013).

Equation	MO	NG	ES
Survival; *logit(s)* =*a* + *bx*			
a	0.43 (0.16)	15.66 (7.45)	1.261 (0.18)
b		-0.26 (0.12)	
Growth; *y* = *a* + *bx*			
a	42.89 (2.35)	36.18 (4.19)	26.89 (2.40)
b	0.36 (0.04)	0.42 (0.07)	0.59 (0.04)
SD	1.97	1.65	1.60
Reproduction; $\Psi = a + bx$			
a	-8.10 (4.05)	0.81 (0.19)	-8.27 (4.71)
b	0.14 (0.06)		0.14 (0.07)
Inheritance; *y* = *a*			
a		29.33 (2.57)	

Table 2. Statistical models describing the demography of *Podarcis lilfordi* in MO, NG and ES. Model selection was done on basis AIC values. *a* and *b* values represent model parameters and standard errors are given in brackets. SD is the standard deviation of the residuals of the growth model. Only statistically significant effects are retained.

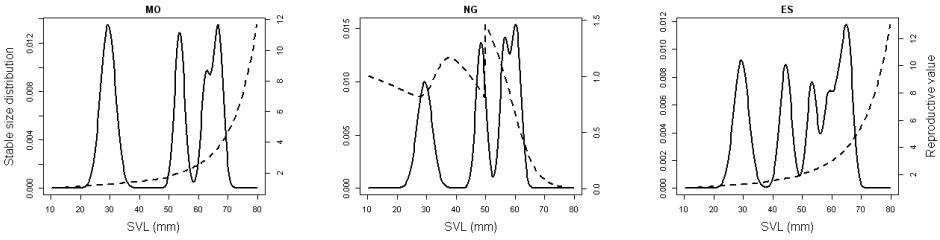

Fig. 2. Stable size distribution (black line) and reproductive value (dashed line) feom the integral projection model in three populations (see text for details).

Size-dependent distribution and population growth rate

The size-dependent distribution appeared similar in all islets but not identical probably due to the difference in growth rate (Fig 2). The size structure in MO was characterized by a high proportion of small (newborns) and large lizards, while intermediate sizes were little represented compared to the other two islets. All populations had a large proportion on new recruits and mature individuals. The reproductive value of NG reminded constant until reaching large sizes and it decreased afterwards (Fig 2). Populations with large sized females had similar growth rate ($\lambda_{MO} = 0.97$, $\lambda_{ES} = 1.03$) while in NG, where females were smaller, it was higher ($\lambda_{NG} = 1.11$).

Elasticity analysis

In MO and ES where lizards are larger, the number of recruits increased for large females, while in NG it showed the opposite pattern (Fig 3). Survival-growth components had the largest elasticity in MO and ES (79% and 87%, respectively) compared with reproduction (21% and 13% respectively). However, in NG the elasticity of the reproduction component was the greatest (28%). This reflects the low reproductive value of large (old) individuals due to the low survival probability (Fig. 4). In MO and ES the contribution of medium-size individuals to population growth is small (Fig 4). Surprisingly, ES shows two peaks one on lager individuals and another in medium-sized individuals, probably due to the slightly higher survival rate that have medium-sized individuals compared to the other lizards in this population. Finally, the size at first reproduction (individuals from 50 to 55mm) is a very important trait in NG unlike MO and ES where it seems to have little influence (Fig 4).

Discussion

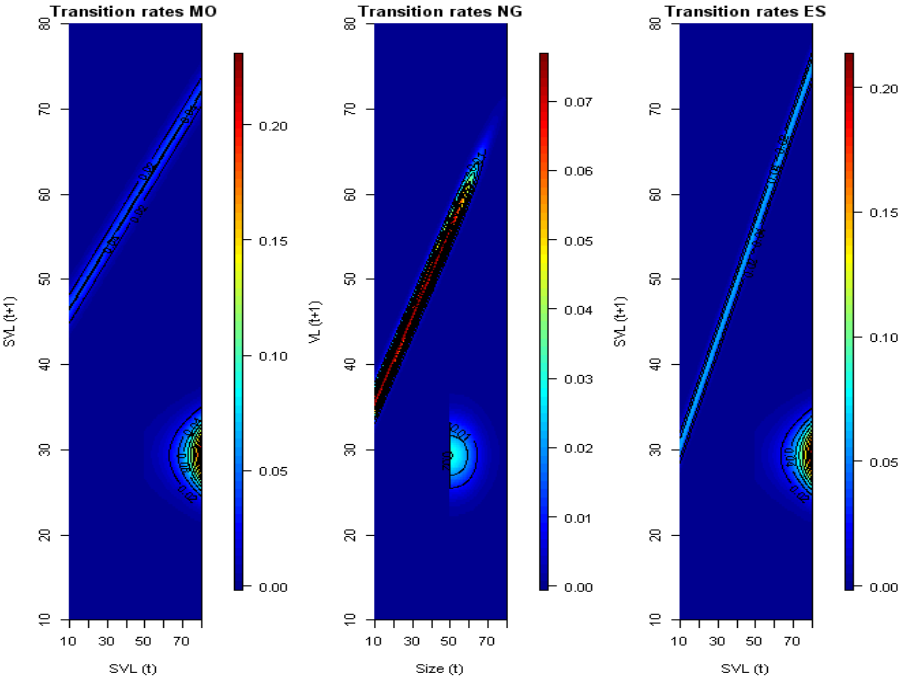

Fig. 3. The transition surface of the integral projection model for the three islets. The x and y axes represent the snout to vent length (SVL) of individuals at time *t* and time *t*+1, respectively; the diagonal indicates the survival and growth of individuals from hatchlings (on the left) to mature individuals (on the right). The half circle along the bottom of the diagonal indicates the number of hatchlings produced by females of increasing size. Contour lines are the surface values of transition rates of individuals between size classes.

We investigated the role of body size in the life-history tactics of Lilford's lizards estimating size-dependent vital rates in three isolated and neighboring populations. Where individual body sizes were large (MO and ES, average adult SVL: 65mm and 67mm, respectively), we found a positive relationship between female body size and the number of eggs produced (see alsoSinervo 1990). However, the number of eggs was independent of female size in the third islet where females were 15%

smaller (NG average SVL: 58mm). The survival probability showed the opposite pattern, being size-independent on the two islands with the largest individuals (MO and ES) while negatively associated with SVL in the one where average size of females was small (NG). The elasticity analysis using demographic models (IPM; Easterling et al. 2000) built on these size-dependent relationships indicated a contrasting role of body size in the three populations. Large females in MO and ES showed the highest expected fitness whereas the marked mortality in large sizes in NG results in a selective advantage of small (young) females. At present it is not clear which mechanism is responsible for a high mortality in large classes in NG. Blanckenhorn (2000) listed four major possible costs associated for being large: 1) viability costs of juvenile development, 2) high predation/starvation probability of larger sizes, 3) decrease mating success of large males and 4) decrease reproductive success of large females due to late reproduction. In our case, juvenile development, i.e. growth, does not seem to generate a cost for larger size. Rotger et al. (2016) showed that juveniles' growth is under the influence of environmental conditions, but not the one of breeding animals. A variable growth rate is not expected to generate a fixed cost for all cohorts.

Bassar *et al*. (2013) showed that predation pressure caused morphological change in guppies *Poecilia reticulata*. In our case a different predatory pressure or level of competition across the populations seems an improbable explanation for the change in SVL because all three islets have the same predatory level and Lilford's lizard is the only lizard species present. Another possible explanation is that larger sizes in NG are too expensive to maintain. Wikelski 2005 (2005) reported that the body size differences in two insular populations of marine iguanas *Amblyrhynchus cristatus* was due by the energetic costs of maintaining larger sizes and the lower food availability in one of the two island. Food limitation seems improbable in our case. Also, according to this hypothesis the smaller lizards should be found in ES, the islet with the smallest surface, the

smallest number of plant species and the highest population density. Results on growth rate, a measure directly related with resources availability (Andrews 1976; Blanckenhorn 2000), are also against the hypothesis that population in NG is food limited, as the lowest growth rate is in ES (See supplementary data). The absence of a SVL-related fecundity in NG suggests a high reproductive investment by small (young) females. This early investment might have a pleiotropic effect on the mortality at older ages (Hillesheim & Stearns 1992; Blanckenhorn 2000). Whatever causes the senescence in NG the low reproductive value of large animals seems to constrain the evolution toward larger sizes in this islet. A limitation of our work is that conclusions are drawn on correlative relationships between SVL and demographic parameters. Correlative studies cannot fully explore the mechanisms underlying the contrasting role of body size (but see Coulson 2012) and, in this respect, our work is not conclusive. In our case, ecological characteristics seem to be an unlikely explanation for the differences found. A possible explanation to the adaptive significance of small size in NG is a selective pressure against large size in historical times. Pregill (1986) showed that in small human-populated islands there has been a selection against large sizes and NG is the only islands with a documented human presence in pre-roman times (c. 700bc-250bc; Guerrero 2000). At present this is a plausible hypothesis, however, speculation and further experiment should be conducted to elucidate the mechanism underlying the contrasting patterns of high mortality in large sizes we have found. Though its limitations, to our knowledge this is the first work that describes the different role of body size in three neighboring populations of reptiles using size-dependent demography.

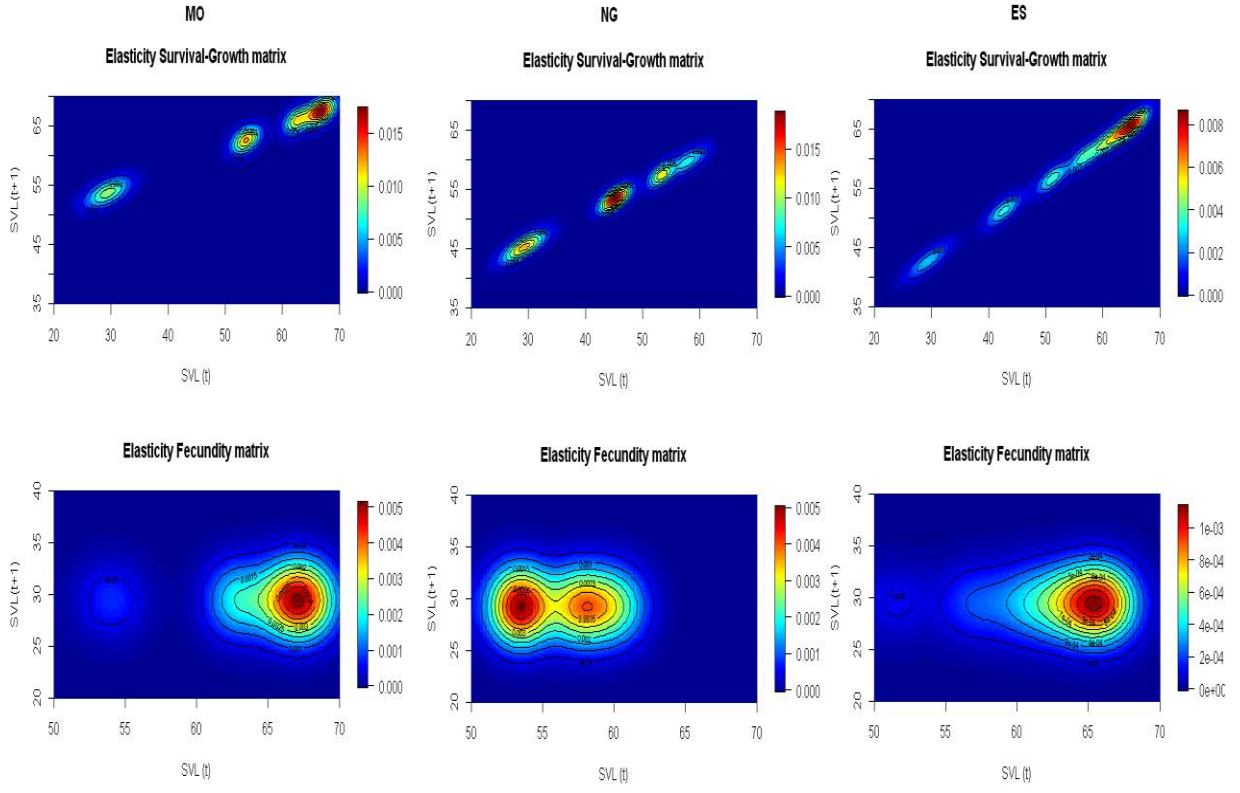

Figure 4. Survival-growth and fecundity kernels elasticities according to islet.

Data Accessibility

The data sets used in this study will be published online by the Mediterranean Institute of Advanced Studies (CEDAI: IMEDEA Data Center; http://cedai.imedea.uib-csic.es/)

Acknowledgements

We are grateful to the members of the Population Ecology Group at IMEDEA. We would also like to thank the Port at Colonia St. Jordi, Guillermo from 'Excursion Boat' and L. Bonnet for their logistic support, the Conselleria de Medi Ambient of the Balearic government for the permission of handling animals. We specially thank Dr. Tim Coulson and Dra. Sonya Clegg, their help were crucial to understand IPMs and learn more about island's theory in my short stay in Oxford. AR was supported by a JAE fellowship from the Spanish Research Council (CSIC, Spain). The authors have no conflicts of interest to declare.

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Supporting Information

Length-at-age curve and state classes

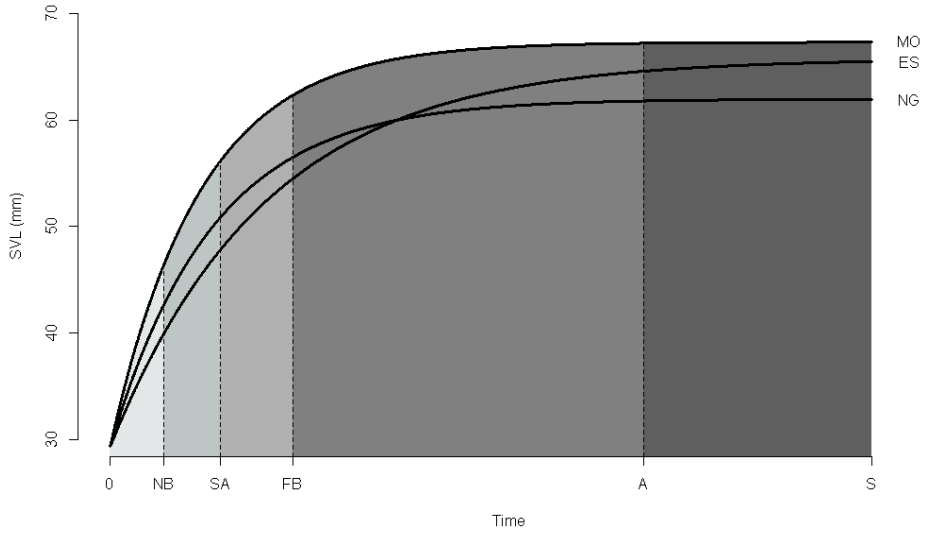
A difficulty or our study was that the age of individuals is unknown. As a consequence it was not possible to directly relate individual size with a life-cycle stage. To approximate a size-to-stage relationship, we considered 5 classes described with the aid of growth models. We used SVL measures collected from June 2009 to October 2015 to estimate the length-at-age curve in females at each population. Rotger et al. (2016) compared different growth equation and found that the Schnute's growth model (Schnute, 1981; Baker et al. 1991) described Lilford's lizard growth adequately, as:

$$L_2 = L_1 \cdot e^{-K \cdot D} + (y_2 - y_1 \cdot e^{-K\,(T2-T1)}) \cdot 1 - e^{-K \cdot D} / 1 - e^{-K \cdot (T2-T1)}$$

where y_1 is the minimum size observed at a given age (T_1) in the sample and y_2 corresponds to the maximum size observed at a given age (T_2 ; being analogue to the asymptotic growth rate), *K* is the characteristic growth parameter and D is the interval of time between recaptures (Fabens 1965; Schoener & Schoener 1978). To account for individual variability in y_2 and *K* individual identity was used as random effects. Schnute model was implemented using nonlinear mixed-effects model ("nlme" package; Pinheiro and Bates 2008). We included population as a factorial explanatory variable (3 levels, MO, NG and ES) and used Akaike's information criterion (AIC) to select the most parsimonious model (Burnham and Anderson 2004). Furthermore, we performed diagnostics tests to asses the heterocedasticity of residuals and the overparameterization of random effects (Pinheiro and Bates 2000). The model with the lowest AIC model was the most general one, assuming a different asymptotic size and characteristic growth for each population (Table S1). We used the estimates from this model to determine the five state-classes which were used to perform the survival analysis (Fig. S1).

Schnute model	AIC			
Fixed effects:			y_2	K
		MO	67.32(0.31)	0.00232(0.0002)
$y_2 \sim Pop, K \sim Pop$	**5056.95**	NG	61.96(0.41)	0.00204(0.0003)
		ES	65.51(0.23)	0.00134(0.0001)
$y_2 \sim Pop, K \sim 1$	5105.20			
$y_2 \sim 1, K \sim Pop$	5163.49			
$y_2 \sim 1, K \sim 1$	5340.29			

Table S1. Growth models for individuals of unknown age using Schnute growth equations. Showing mean values of asymptotic body size and characteristic growth rate of the best model (in bold). AIC, Akaike's information criterion. Pop, covariate population in fixed effects (MO, NG, and ES). y_2 asymptotic body size (mm). K, characteristic growth rate (day^{-1}). SE in brackets.



Figures S2. Estimated growth curve with the five state-classes used in the survival analysis. Each color represents a state-class. NB, newborn; SA, sub-adult; FB, first breeder; A, adult; and S, senior.

Goodness of fit

Capture-recapture models are based on several assumptions. The most important of them is that future survival or recapture probabilities do not depend on the events occurred at a given capture-recapture session. That is to say, newly-marked individuals should have the same survival probability of already-marked ones and the current recapture probability is not influenced by the past capture history (Tavecchia et al. 2008). Multistate models for stratified data have additional assumptions on between-state transitions (Pradel et al., 2003). These assumptions can be assessed using contingency tests built under the hypothesis that all assumptions hold with software U_CARE (Choquet et al. 2009l).

	MO			NG			ES		
Test	χ^2	df	*p-value*	χ^2	df	*p-value*	χ^2	df	*p-value*
3G: New Captures=Old captures:									
3G.SR (Transients)	6.898	12	0.860	5.965	6	0.427	10.489	11	0.487
3G.Sm (Memory)	8.664	20	0.986	1.872	7	0.967	16.446	21	0.744
WBWA: before vs. after	0	2	1	0	1	1	1.344	5	0.930
M: ITEC+LTEC	2.139	2	0.343	0	0	0	1.335	2	0.513
Total (3G + M)	17.701	36	0.995	7.837	14	0.899	29.614	39	0.861

Table S2. Summary of U-CARE test results for the three study populations. Tests 3G and M added up to the global goodness-of-fit test. Test statistics (χ2), corresponding degrees of freedom (df) and p-values are given. See Choquet et al. (2009) for more details on test compon

General Discussion

Ecologists and evolutionary biologists aim to understand the processes that determine the diversity and distribution of organisms and the way they interact with the environment. Under the fast pace of global changes, the speed at which individuals respond to environmental changes has become an issue of particular interest, as a consequence much attention has been devoted to investigate the times-scale of ecological and evolutionary responses. Recent evidence about phenotypic and genetic changes caused by human-related selective pressures suggested that evolutionary responses occur faster than previously thought. The fast responses helped to fuse ecological and evolutionary views, concepts and methodologies. The unified approach of eco-evolution is a rapidly growing field merging approaches once considered exclusive of single disciplines such as genetic, ecology, evolution or statistics (Hairston *et al.* 2005; Fussmann *et al.* 2007; Ezard *et al.* 2009; Pelletier *et al.* 2009). Based on multiple approaches, eco-evolutionary studies are data demanding, and despite a strong theoretical framework (Fussmann et al., 2007), there is a lack of empirical studies that fill the gap between data and theoretical predictions in many levels of biological organizations (Whitham *et al.* 2006; Kasada, Yamamichi & Yoshida 2014). For instance, empirical studies on individual plasticity in natural populations are relatively rare despite the importance of plasticity in modulating individual response to environmental changes (but see Nussey, Wilson, & Brommer, 2007). An important reason behind the lack of empirical eco-evolutionary studies is the difficulty of the long-term monitoring of individuals' traits and life-history data. In this thesis I integrated ecological, evolutionary and genetic approaches to investigate life-history strategies in three insular lizard populations. Most studies on

eco-evolutionary processes are carried out in laboratories or controlled and semi-natural systems, e.g. mesocosms (Bassar *et al.* 2015). However, in laboratories or in mesocosms, natural variability is by definition absent and selective pressures are only those imposed by the researchers. Population density, age-structure, food accessibility and predatory pressure, for example, are not reproduced in most laboratory experiments. On the other hand, monitoring individuals in natural conditions is difficult and part of the information is inevitably missed. Furthermore, each population is unique and has a particular age and sex-structure that may change or fluctuate across the time and this structure will rule the dynamics of the population. This demographic context is an important agent of eco-evolutionary feedback that should be considered, in light of the speed of evolutionary changes. During my study of *Podarcis lilfordi* I investigated a reliable way to sex, age and recognize individuals from the different populations (Chapters I, II and III). I then used the gathered information to address eco-evolutionary issues on individual growth (Chapter II and III) to investigate the role of body size in lizards' life-history tactics by comparing the functional relationship between SVL and demographic traits across three populations (Chapter IV). Finally, I used the genetic information to inferred population histories and potential past selective pressures (Chapter IV).

The methodological advances

During my thesis I needed to overcome some methodological obstacles linked with individual recognition and population surveys to obtain reliable data to address biological questions. I briefly discuss below the major issues of these methodological advances.

Individual recognition: a new non-invasive technique

Individual marking allowed important insights in animal ecology and evolution (Lebreton & North 1993). However depending on the type,

marks can negatively affect the behavior and the survival of marked individuals. For example, flipper bands used on penguins have been found to increase the mortality rate within the population (Culik, Wilson & Bannasch 1993). This negative effect is even more pronounced in the study of amphibians and reptiles. Amphibian and reptiles, especially lizards, were typically marked by toe-clipping (May 2004). However, McCarthy & Parris (2004) showed how mortality increased linearly with the number of toes clipped. This type of marking is now no longer recommended (May 2004). The use of the customized software, APHIS (Automated Photo-Identification Suite; Moya et al. 2015), permitted to successfully identify individuals from a digital image of the ventral scales (see chapter I.I).

Fig. 1. Matching between a sample image (left) and one of the possible candidates (right), classified according to the match score.

I gathered a total of 5000 images from an average of 800 individual per populations. Even with such a large repository, APHIS was able to correctly identify new images from already known animals.

Estimation of Population size

Population size is a central parameter in ecological studies, however in natural populations it is impossible to carry out exhaustive counts. Many analytical and methodological tools have been developed to solve this problem. Method election to estimate population size is important because it has assumption to fulfil, and often depends of the type of study's population (opens or closed) and species (size, behaviour). The soft comparison of density by different sampling methods was useful to obtain an accurate estimate of population size and select the most suitable method to study insular lizard populations. Although with comparable results, Line-Transect (LT) method was the method that resulted in largest variance of the estimate due to variation among habitats and the inevitable departures from model assumption. Capture-Mark-Recapture (CMR) method without the spatial information provided estimates with small variance but only in multiple sessions. In contrast we showed that Spatial Explicit Capture Recapture (SECR) models delivered estimates that provided a variancem, which was independent of the number of occasions. Therefore, SECR is a better method when the number of occasions is limited, whereas CMR is more accurate whether the number of occasions is frequent. From the other side, LT is suitable when the habitat is more uniform or with less mobile species. Adult Balearic wall lizards (*P. lilfordi)* can be sexed at capture by visual inspection of femoral pores in males (Dellinger et al., 1990). However, juveniles (aprox. <50mm), and sometimes older lizards, cannot be reliably sexed. Lecomte (1992) suggested that the number of scale rows can be different in males and females and can be used to sex juvenile animals.

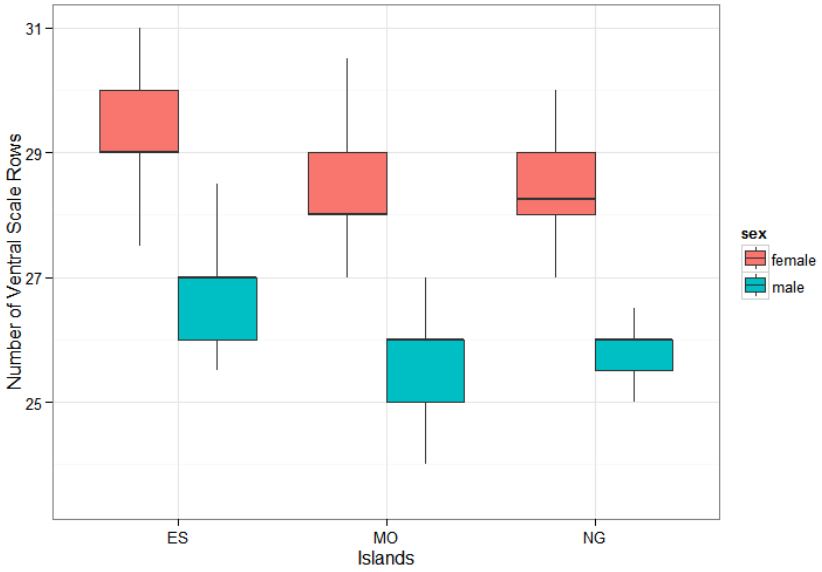


Fig. 2. Number of rows scales in males and females of three populations.

In *Podarcis lilfordi* Salvador (1980) showed that females in Cabrera archipelago had on average more ventral scale rows than males but this difference changed across populations. I first assessed the difference in row numbers of ventral scales for each population using known males and females. We use scale row number as to sex most individuals. (Lecomte, Clobert & Massot 1992). Male lizards had on average fewer ventral scale rows than females (See Chapter II). Longitudinal analysis indicated that scale count remained constant through an individual's lifespan. Lizards with more than 28 scale rows were always females and those with fewer than 26 were always males (Fig. 2). It is likely that the extra row scale of females is due to the need for space during egg development. With this 'personalized' methodology for each population it was possible to sex around 75% of previously unsexed individuals. We showed that each

population is different and generalizations on the discriminant power of scale row are difficult.

Fig. 3. Differences in body size between juvenile individual (left) and an adult male (right)

In natural population, unless animals are marked at birth, individual age is unknown. Therefore, relating individual size with a life-cycle stage is very difficult. Long-term capture-recapture data (see chapter I) were used to estimate the length-at-age growth curve (Schnute, 1981; Baker et al. 1991, Somers 2004) and the age of sexual maturity for both sexes (chapter II) and among populations (chapter III). The data-rich sample from Na Moltona islet showed that an oscillatory version of the Schunte model

best described the growth data (Appeldoorn 1987). This suggests that the growth rate abates during the winter period. The slow winter growth is in agreement with the size-structure of the population in April, which missed an intermediate class (see Chapter IV). It also indicates that lizards born in late summer will probably reach sexual maturity older than one year old, increasing fitness costs for late reproducing mothers. The comparison between the three islets has been on the Schnute's model. Growth parameters varied over sex as found in other lizard species. Interestingly, I found statistically significant differences among islets. ES was the islet with the lowest characteristic growth (*K*), and NG, along with MO, had the highest value of *K*. Food availability may be responsible of the lower *K* in ES (see below for discussion). Using the estimates from this growth model, along with previous ecological knowledge about the species, I established differentiated state-age-classes which later were used to perform a survival analysis (see chapter IV) and characterized size-dependent life cycle.

Evolutionary demography of the Balearic lizard

The island syndrome and lizard body size

A recent review showed an inconsistent pattern of body size on island, with some large-medium species becoming smaller than predicted by the island sydrome (Meri 2007). A consequence of this is that some authors questioned whether islands are food limited habitats (Meiri 2007; McNab 2010; Raia *et al*. 2010). Andrews (1976) pointed out that food availability on islands might be variable and that lizard growth in food-limited islands was slower than mainland population or than other islands with more resources. Our data support the Andrews' (1976) argument, being the lowest characteristic growth (*K*) in the smallest and food-limited population ES (Chapter III). This indicates that growth is limited by resource availability but this limitation does not prevent individuals to reach large sizes. It appears that the two main parameters of the growth

curve, K and L_{inf}, are under different ecological drivers. While K is under the control of resource availability, the asymptotic size, L_{inf}, seems to depend on life-history tactics and intra-sexual competition. Although, both parameters are under different selective pressures, the two parameters are not fully independent.

Comparing our findings with those in other *Podarcis* populations both in mainland and islands, we found that island lizards grow slower than their mainland counterparts. This 'island effect' is also supported by the difference within the same species (Table. 2). Insular *P. hispanica* and *P. taurica*, for example, grow slower than their mainland conspecifics despite having a larger asymptotic SVL. On the other hand, the difference of K values between the smallest and the largest species observed is more pronounced within islands than in mainland species (island: K_{SVLmax}-K_{SVLmin} = 0.0012 days^{-1}, mainland: K_{SVLmax}-K_{SVLmin} = 0.0003 days^{-1}). The largest range on islands reflects a higher sensitivity of K-values to environmental conditions in these isolated populations. Therefore, as Meiri (2008) suggested, lizards do not follow the island syndrome as first thought because, even though insular populations are 10 times denser than mainland populations (Buckley & Jetz 2007), size is not affected and large species become even larger on islands, only that insular lizards need more time to reach it.

In species with continuous growth like reptiles density-dependent and -independent factors have been shown to influence individual growth rate during their entire life-cycle, but their relative role differed according to individual age or sex (Mugabo *et al.* 2010, 2011, 2013). LeGaillard et al (2010) showed that an environmental-dependent growth rate was the mechanism underlying the differences among cohorts. However, in chapter III it was also added density as an important factor to take into account suggesting that a complex interplay between extrinsic (climatic) and intrinsic (density) factors govern over the variability of body growth

rate. The different roles according to stage of individual generate compensatory responses, for instance a bad year for juveniles due to a hot summer will be compensated by a faster growth in yearlings.

Genetic divergences among islands, survival and fecundity differences across the islands

Besides the differences found in growth parameters between island and mainland populations and the effects of density and climatic factors on growth rate in different ages of a population, the divergences observed in our three populations of study in life history traits were still large, considering that three populations are of the same sub-species (Salvador 1986) and belong to the same genetic clade (Terrasa *et al.* 2009). When I delved further into their life history traits such as survival rate and fecundity, I found significant differences among populations. First of the population of NG is composed by individuals (males and females) 10 mm smaller than those in the other two island. Body size is an important driver of life-history traits and its importance can inform on the variability of selective pressures (Peters 1986). Therefore, the difference in size among populations may influence survival and fecundity rate.

Species	T	L0	L	Lmax	K	Habitat	Locality	References
Podarcis atrata	270	29	51	71	0.00275	island	Columbretes	(Keller & Waller, 2002; Lacy, 1997)
Podarcis hispanica	210	24	42.2	68	0.00254	island	Cíes island	(Bauwens & Diaz-Uriarte 1997)
Podarcis lilfordi	360	31.9	50*	70	0.0018	island	Cabrera	Castilla and Bauwens (2000)
Podarcis milensis	420*	25*	42	63	0.00141	island	Milos island	(Adamopoulou, Valakos & Price 2000)
Podarcis pytiusensis	390	27.8	49	68	0.00192	island	Ibiza island	(Carretero *et al*. 1995)
Podarcis taurica	270	30*	52.2	74	0.00260	island	Ionian islands	(Chondropoulos & Lykakis 1983)
Podarcis bocagei	240	20	44	64	0.00329	mainland	Coruña	(Bauwens & Diaz-Uriarte 1997)
Podarcis erhardii	250	27.8	48	66	0.00301	mainland	Greek mainland	Maragou et al. (1999)
Podarcis hispanica	240	27	49	67	0.00333	mainland	Salamanca	Bauwens and Diaz-Uriarte (1997)
Podarcis hispanica	240	20	39	57	0.00300	mainland	Asturias	(Bauwens & Diaz-Uriarte 1997)
Podarcis muralis	300	24.1	49	65	0.00313	mainland	Asturias	(Bauwens & Diaz-Uriarte 1997)
Podarcis peloponnesiaca	270	28.2	60	82	0.00331	mainland	Peloponnisos	Maragou et al. (1999)
Podarcis taurica	300	26.7	52.5	70	0.00302	mainland	Serbia	(Ljubisavljević, Džukić & Kalezić 2010)

Table 1. Reproductive parameters of female *Podarcis* lizards in the field, T is the age at sexual maturity in days, L0 is hatchling size in mm snout-vent length, L is the smallest size of a reproductive female, Lmax is maximum size in mm snout-vent length and K is the characteristic growth rate of the Von Bertalanffy equation. *data were obtained directly from the authors. The estimates of our best growth model are in bold

In species with continuous growth like reptiles density-dependent and -independent factors have been shown to influence individual growth rate during their entire life-cycle, but their relative role differed according to individual age or sex (Van Valen 1965; Andrews 1976; Ballinger & Congdon 1980; Massot *et al*. 1992; Coulson *et al*. 2001; Galliard *et al*. 2010; Mugabo *et al*. 2010, 2011, 2013). LeGaillard et al (2010) showed that an environmental-dependent growth rate was the mechanism underlying the differences among cohorts. However, in chapter III it was also added density as an important factor to take into account suggesting that a complex interplay between extrinsic (climatic) and intrinsic (density) factors govern over the variability of body growth rate. The different roles according to stage of individual generate compensatory responses, for instance a bad year for juveniles due to a hot summer will be compensated by a faster growth in yearlings.

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NG has the lowest survival rate for both males and females in the three populations (chapter III). However, NG is neither the smallest and food-limited nor the densest populations, two factors that may affect on the survival rate. Surprisingly, ES is characterised by a poorer floristic composition and a high dense population compared with the other two populations that had the highest annual survival probability, followed by MO, the biggest and less dense island. Reproductive traits are a "must-have" parameter to carry out eco-evolutionary studies. However, it is also one of the most complicated to obtain for small vertebrates. Moreover complexity increases when carrying out these studies on a polygamistic species such as these lizards. We used an experimental approach to estimate lizard fecundity (Fig. 4, chapter III and IV).

Fig. 4. Animal facilities. Eggs with different sizes and a individual hatching.

The fecundity analysis showed that females of MO laid more eggs than in ES and NG, however, females from NG laid eggs at smaller sizes, a possible explanation may be females from NG reach sexual maturity in smaller sizes, further the number of eggs laid in NG was constant and it did not vary with size (chapter IV). Females from NG may have a high reproductive investment at younger age. Is this younger investment in reproduction the cause of high mortality in NG or is to be ascribed to a

genetic component? Lizards show a remarkable degree of morphological differentiation and life history traits among these three populations and the causes of this divergence may be diverse. Using 15 neutral markers I found out that the genetic divergence among the three populations does not match either the morphological or the ecological differences among the islets (Chapter III). Genetic results revealed an isolation-by-distance effect where ES, the farthest population, was the most differentiated genetically. In fact microsatellite data coupled NG and MO as single cluster. Although NG belongs to the same genetic pool that MO, allele diversity of NG is much lower. High levels of inbreeding may explain low survival rates (Lacy 1997, kleller 2002). In these population levels of inbreeding were normal (chapter III), moreover it was ES the one that had the highest inbreeding level and such inbreeding was correlated to the islet's area. The most striking result was the low number of private alleles in NG (0.7) compared with the other two islets (MO and ES). Simulation analysis integrating different plausible scenarios (see Chapter IV) suggested an important reduction in the effective population size dating back about 620 generations (c. 2200 years before present). This coincides with the date of human settlement in the island (about 400 BC; Guerrero, 2000). The influence of past human settlement may influence and change selective pressures in NG, the introduction of predators by this settlers may select smallest individual forcing population to reproduce early provoking that the investment of early reproduction affect survival in older individuals, specially females.

Tracking the dynamics of body size

Individuals in NG were on average 10-15% smaller than in other islets. Despite the cause for this reduction has not been proved directly, coalescence analysis suggested that this might have resulted from the presence of humans in NG in historical time. A reduction in body size has probably trigged or it has been linked with other life-history trait shifts. To investigate this it was necessary to measure the role of body size within

the lizard life-cycle through a demographic model in which parameters were expressed as a function of body size. The demographic model was instrumental in showing the different ecological strategy followed by NG (Integral Projection Models, see chapter IV). While fertility is constant during the lifespan it seems that mortality increased with female size (and age) in NG. The opposite pattern was found in MO and ES, where larger lizards seemed to have a fitness advantage due to the positive effect of size on fecundity and the apparent absence of as a survival cost. Elasticity analysis confirmed that growth and survival were the most important traits in MO and ES favoring selection to have larger females in the system, and even though the larger the females the more eggs are laid; body size is limited by senescence of the older individual. . In contrast in NG selection is leading by the fertility at small sizes that seems to compensate the mortality of large sizes. Population growth in three populations was quite stable ($\lambda \approx 1$), having NG the highest value (1.12), this means population grows 12%. The investment of early reproduction and constant fecundity increase the population growth in spite of the high mortality suffered by the large individuals. Selection will therefore favor the small body sizes in the case of NG.

Note the importance to consider the effects of current as well as past human presence as an additional evolutionary force shaping natural populations. This thesis shows as a possible explanation the effects of a historical human settlement that may alter the life history traits of an insular population. However, this hypothesis has not been evaluated directly. What is shown directly here is how both evolutionary and demographic processes have affected individual responses differently causing rapid selection. Natural selection acts fast and may take different directions; therefore it is necessary to integrate fine-scale information on the ecology and demographic context for the study of these fast evolutionary changes.

Conclusions

1. Individual marking allowed us to collect detailed information to address question on population ecology, evolutionary and demographic studies. Possible classical marking methods are slowly replaced by new non-invasive approaches for individual identification. The development of APHIS has allowed us to achieve a large and reliable dataset of individual-based information through capture histories in a semi-automatic routine avoiding the classical toe-clipping.
2. Models that took into account individual home range and movements provided a reliable tool to estimate population sizes. They delivered precise estimates regardless the number of occasions. Line-transects and classical capture-recapture methods with no spatial information delivered estimates of population size with large variance and variable accuracy.
3. Growth parameters were different between sexes and among populations. Males reached larger size than females in all populations, but sexual size dimorphism changed according to the population considered. Animals reached asymptotic size slower in the smallest island suggesting that the characteristic growth parameter depended on food availability. Growth parameters had consequences on sexual maturity and survival of the individuals suggesting tradeoffs between growth, survival and reproduction.
4. Although, density in insular habitats is larger than mainland, body size is not significantly affected by insular environment as in the case of mammal or bird populations. Moreover, characteristic growth showed a remarkable difference between island and mainland populations due to the resources-limited of many islands.

5. Despotic competition in insular lizard populations strongly affects life history traits of juveniles. Growth rate of juveniles is limited by high density of population and also by lack of rainfall. Water constraints affected yearling individuals as well. Being adult-sized individuals are the most benefited when conditions are not well. This interaction between covariates and size is probably the reason for the variable temporal size-structure of the populations.
6. The study of the genetic structure of the three islets revealed an effect of isolation-by-distance, the farthest population being the most genetically different. Moreover, the populations of MO and NG belong to the same genetic pool, although NG has a lesser allelic richness.
7. The use of inference Bayesian programs has been instrumental in defining an important reduction population size in NG population. The timing of this reduction was carried out matched with a historical human settlement in NG during 400 years 2500 years ago. Although these results are not conclusive, they stress the importance of accounting for the human imprint in evolutionary and ecological studies.
8. Largest females in NG suffered a high mortality compared to large females in other islets. In NG females seemed to compensate this high mortality with an early reproduction.
9. Elasticity analysis showed a contrasting role of body size in the three populations. Large females in ES and MO have a selective advantage, while in NG the fecundity of small reproductive females is important for the population growth.
10. The three insular populations showed a high degree of life history traits, and genetic divergence. These differences are likely the final result of the particular selection processes that each population has endured.

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Resum de la tesi

INTRODUCCIÓ GENERAL

1. El naixement de l'ecologia evolutiva

Aquests darrers anys hi ha hagut un interès cada vegada més gran per part dels biòlegs evolutius en intentar entendre com els individus interactuen tant en el mitjà abiòtic com biòtica, incorporant així conceptes típics d'ecologia (Fox *et al*. 2001). Encara que aquesta unió entre conceptes ecològics i evolutius s'hagi donat de manera conscient des de fa relativament poc temps, els primers estudis integrant ambdues visions sorgiren a mitjans de segle XX, sent un dels exemples més tipics el del canvi de freqüència del melanisme a les arnes (Biston bitularia) per l'ennegriment de les escorces dels arbres degut a revolució industrial (Kettlewell *et al*. 1971). Més exemples varen venir després qüestionant el fet que l'evolució no passava tan lentament com es pensava (Carroll *et al*. 2007). De fet totes les evidències apuntaven que l'ecologia i l'evolució esta molt relacionades en tots els processos biològics i que a més ocorren a la mateixa escala de temps. Tot això va donar lloc al naixement de l'ecologia evolutiva (Pelletier *et al*. 2009).

Un dels principals problemes que va fer que el sorgiment d'aquesta nova disciplina fos tan tardana va ser perquè encara no hi havia definit cap marc teòric i analític per poder mesurar aquests canvis, gràcies a l'arribada dels nous avanços tecnològics, genètics i computacionals van ajudar a consolidar aquest nou camp (Hairston *et al*. 2005). Així, es va poder confirmar que hi ha unes interaccions recíproques entre la selecció natural (dinàmica evolutiva) i canvis ambientals (dinàmica ecològica)

(Schoener 2011) responsables a modelar i mantenir la biodiversitat (Post & Palkovacs 2009).

L'interès d'aquestes interaccions és estudiar les contribucions relatives entre l'ecologia i l'evolució a les dinàmiques poblacionals i són el resultat de forces naturals o antropogèniques (Lankau & Strauss 2007; Pelletier *et al*. 2009; Ellner *et al*. 2011). Aquesta interacció dinàmica també va ser anomenada per Turcotte, Reznick & Hare (2013) com a *feedbacks* eco-evolutius. Aquests *feedbacks* afecten múltiples nivells d'organització biològica des dels gens fins als ecosistemes (Bailey *et al*. 2009). És a dir, els efectes de la variació genètica o fenotípica tenen conseqüències a nivell de la població, i aquesta afectarà la comunitat i en darrer lloc a l'ecosistema. Així, per exemple, els individus amb els trets més avantatjosos podran sobreviure millor i reproduir-se passant els seus gens a la pròxima generació canviant així les freqüències de distribució dintre la població. Aquesta influencia sobre el tret seleccionat, pot tenir un efecte directe o indirecta sobre l'estructura de la comunitat i finalment sobre l'ecosistema. Un bon exemple d'això va ser proporcionat per Palkovacs *et al*. (2009) que va mostrar que els guppies *(Poedilia reticulata)* canviaven els seus trets d'història de vida d'acord al nivell de predació, aquest fet afectava directament a la producció de biomassa per part de les algues sent més alta quan hi havia molta depredació afectant tota la comunitat.

Tots aquests canvis són molt ràpids, especialment en espècies amb un cicle de vida curt i poden passar en unes poques generacions (Hairston *et al*. 2005; Carroll *et al*. 2007). Això fa que la selecció fluctuï dins d' escales de temps ecològiques generant canvis evolutius, a aquest procés també s'anomena evolució contemporània (Hendry i Kinnison 1999; Kinnison i Hendry 2001). No obstant això, cal tenir en compte els canvis en el passat perquè l'ocurrència dels processos en el passat han donat forma a la diversitat que es veu avui en dia. Encara que no és possible investigar

aquests processos passats directament, sí que es pot explorar possibles escenaris anteriors i una ampla varietat de patrons evolutius tant, abiòtics (fluctuacions climàtiques, augment o disminució del nivell de la mar) com biòtics (depredació històrica) del passat. En un context filogenètic, el mètode comparatiu s'utilitza per obtenir una idea de trajectòries històriques que condueixen a patrons contemporanis. Un dels exemples més importants dels estudis de l'evolució en el passat utilitzant el mètode comparatiu va ser per Losos (2009) amb el seu estudi sobre la morfologia dels anolis; la seva ecologia i la radiació adaptativa històrica dels individus a les diferents illes; a més d'utilitzar l'enfocament filogenètic per explorar els patrons de coevolució de la morfologia, la fisiologia i el rendiment locomotor; va integrar també les interaccions recíproques dels patrons històrics (evolució) i els processos actuals que poden produir aquest patró (ecologia).

2. La demografia evolutiva i el seguiment de les poblacions silvestres d'animals

La gran variabilitat fenotípica observada en les poblacions animals és el resultat de la combinació dels gens i el medi ambient (Schlichting, Pigliucci i altres 1998). Per tant, les interaccions entre el genotip i l'ambient són especialment rellevants per a l'estudi de la variació fenotípica i selecció natural en les poblacions animals. A escala poblacional els trets fenotípics i la demografia són importants motors dels processos microevolutius (Ezard et al., 2009). *Però com l'evolució influència exactament a la demografia i viceversa?* Se sap que aquest feedback eco-evolutiu sorgeix a causa d'un canvi en l'estat de la població que altera les pressions selectives donant noves distribucions de fenotip, a continuació aquestes distribucions afectaran a la densitat que pot afectar després a tota la distribució posterior del fenotip (Coulson et al., 2006). Per exemple, Hairston et al (2005) va utilitzar una llarga seqüència de dades sobre el bec en el pinsà de Darwin (*Geospiza fortis*) i va mostrar

que la variació de les condicions ecològiques en diferents dècades impacta en la força i direcció de la selecció de la mida del bec. Per tant, sabent que a les dinàmiques de poblacions naturals els processos ecològics i evolutius estan entrellaçats cal desenvolupar mètodes per quantificar la seva relació. Un primer pas és caracteritzar la relació entre la variació fenotípica en què opera la selecció i el creixement de la població.

No obstant això, un dels principals reptes per aquests tipus d'estudi és aconseguir un gran nombre de dades individuals a llarg termini (Clutton-Brock i Sheldon 2010). A més, el seguiment de les poblacions d'animals al llarg del temps per obtenir mesures repetides representa un veritable desafiament pels biòlegs.

Els estudis sobre la demografia evolutiva tenen com a objectiu n'última instància predir la direcció futura de la població i investigar com la població contemporània respondrà als patrons contemporanis de selecció (canvis fenotípics). Un aspecte important per fer front a aquestes preguntes ha estat el recent desenvolupament de l'anomenada genètica quantitativa (Rees i Ellner 2016). Aquesta àrea del coneixement estudia l'heretabilitat dels efectes ecològics dels organismes i les relacions genètiques entre individus.

3. La demografia evolutiva de la sargantana balear (*Podarcis lilfordi*)

Les poblacions naturals tancades i petites com els illots continentals són models biològics adequats per la realització d'estudis eco-evolutius perquè el tancament demogràfic (no migració) i l'escala espacial limitada contribueixen a reduir la complexitat dels processos ecològics. De fet, el marc teòric en el que s'ha desenvolupat l'ecologia i la demografia no seria el que és ara sense els estudis a les illes (Warren et al. 2015). Des de MacArthur i Wilson (1963), les illes han proporcionat nous coneixements a la comprensió dels processos fonamentals de l'ecologia i l'evolució i han

tingut un paper clau en la teoria de l'evolució (Darwin 1859; Lomolino 2005). Donat el context ecològic relativament simple de les illes, les pertorbacions tan humanes com a naturals actuen com una força selectiva important (Pregill 1986; Grayson 2001). No obstant això, hi ha poques evidències i pocs estudis rigorosos de com històricament les activitats humanes han afectat poblacions silvestres. Per exemple, Pregrill (1986) va proposar que els assentaments humans històrics en petites poblacions properes (illots) afectaven directament el fenotip de petits vertebrats mitjançant la selecció en contra de grans mides.

Un altre factor a tenir en compte l'anomenat efecte illa, els individus insulars tenen una sèrie de peculiaritats de comportament i fenotípiques en comparació a les poblacions continentals degut a l'absència de depredadors, la baixa disponibilitat de recursos i la competència inter-específica reduïda (MacArthur, 1967; Roughgarden, 1972; Adler i Levins, 1994). La teoria d'illes prediu que els petits vertebrats a les illes tendirien a augmentar de mida, baixar la fertilitat, sobreviure més temps i arribar a poblacions més denses que els seus parents continentals (Adler & Levin 1994; Palkovacs 2003 ; Buckley i Jetz 2007). No obstant això, a les poblacions illenques de sargantanes no es compleixen totes aquestes assumpcions (Meiri 2007 ;. Raia et al 2010). Per una banda, les poblacions de sargantanes insulars aconsegueixen altes densitats, però la mida del cos sembla no canviar d'acord a les prediccions (Meiri, Cooper i Purvis 2008). De fet, les sargantanes mostren una notable variació en la grandària del cos a través de les seves poblacions, aquesta variabilitat suggereix que la mida corporal és un tret molt plàstic que respon a pressions selectives locals (Meiri 2008).

El gènere *Podarcis* (Wagler 1830) comprèn les sargantanes d'Europa i el nord-oest d'Àfrica, amb aproximadament 23 espècies descrites (Uetz et al. 2016) i prop de 300 subespècies insulars (Böhme, Hutterer i Bings 1985). Aquest grup va evolucionar i es va diversificar a la conca mediterrània (Arnold, Burton i Ovenden 1978). Totes les espècies de

Podarcis són enormement variables i sovint notablement plàstiques des d'un punt de vista fenotípic, tant en la coloració i en la morfologia i el nombre d'escames.

La sargantana balear (*Podarcis lilfordi*, Günther 1874) són sargantanes de grandària mitjana endèmiques de l'arxipèlag de les Illes Balears. L'origen de l'espècie va ser durant la reinundació de la Mediterrània al final del Messinià fa més de 5 milions d'anys (Krijgsman et al 1999 ;. Duggen et al. 2003; Brown et al 2008). *Podarcis lilfordi* només es troba als illots al voltant de Mallorca i Menorca i a l'arxipèlag de Cabrera (Pérez-Mellado, V. & Martínez-Solano, I 2009).

Tot i que el procés evolutiu de la sargantana balear ha estat molt complexa, *Podarcis lilfordi* és una bona espècie per abordar qüestions de demografia i ecologia evolutiva, ja que tots els processos històrics de diferenciació i diversificació estan ben documentats (Terrasa *et al*. 2004, 2009). A més, hi ha evidència de llargs períodes d'incursions humanes en algunes illes que podrien haver generat pressions selectives específiques (Guerrero 1997). A més, moltes poblacions de sargantana balear, a més de compartir una estructura genètica similar, habiten en hàbitats similars però difereixen en la densitat, la coloració, la forma del cos, la mida del cos i els trets d'història de vida (Salvador 1980, 1986). Aquesta variabilitat és la base necessària per fer front a la pregunta sobre el seu manteniment i origen.

Les meves investigacions s'han centrat en tres poblacions aïllades veïnes d'aquesta espècie. Les tres poblacions triades són de la costa sud de l'illa de Mallorca: Na Moltona (en endavant, MO), Na Guàrdia (NG) i Es Curt (ES), les tres poblacions pertanyen al mateix grup genètic derivat del mateix esdeveniment de colonització. S'han utilitzat mètodes comparatius per estudiar la contribució dels processos ecològics, ambientals i evolutius que governen les dinàmiques d'aquests illots

OBJECTIUS

L'objectiu principal d'aquesta tesi ha estat la de posar de manifest les pressions selectives imposades per l'entorn insular i les conseqüències de les respostes individuals en l'evolució dels trets d'història de vida. Al llarg d'aquesta tesi he fet servir tècniques i conceptes de l'ecologia, genètica i morfologia per desenvolupar un model de població integrat que proporcioni un nou marc analític per abordar qüestions sobre dinàmiques eco-evolutives i que unifica els enfocaments ecològics i genètics en un context demogràfic.

Capítol I, seguiment d'animals en poblacions naturals. Aquest capítol es divideix en dues parts corresponents a dues publicacions diferents presentades en aquesta tesi. La primera part està dedicada al desenvolupament de tècniques innovadores no invasives per al reconeixement individual. En particular, he participat en el disseny d'un nou programa, el APHIS (Automated foto-identificació Suite) per a la identificació semiautomàtica de sargantanes a partir de patrons d'escames. L'objectiu de la segona part d'aquest primer capítol està dedicat a identificar les fortaleses i debilitats dels mètodes disponibles per obtenir una estimació no esbiaixada de la densitat de sargantana o mida de la població a través d'una validació acurada dels resultats.

Capítol II, Denso-dependència i independència en la taxa de creixement individual. Aquí, he utilitzat les dades individuals recol·lectades en el camp per estimar l'edat dels individus mitjançant una corba de creixement. Una vegada que es descriu la relació de la talla per edat, quantifiquem el paper relatiu de la densitat de la població i dels factors climàtics que afecten la taxa de creixement a cada classe d'edat.

Capítol III: Genètica de poblacions. Estudi de l'estructura genètica. En aquest capítol he analitzat els patrons de divergència entre les tres poblacions utilitzant marcadors genètics. Una vegada que he establert les

diferències genètiques de les tres poblacions vaig comparar característiques d'història de vida com la mida corporal, la taxa de creixement individual, la fecunditat, i la probabilitat de supervivència. En un illot la mida corporal mitjana va ser aproximadament 14% menor que en els altres dos, sent una possible explicació uns esdeveniments històrics ocorreguts en el passat que poder ser rellevant per a la dinàmica de població d'avui.

Capítol IV: model de projecció integral (IPM). Aquí, he desenvolupat un IPM per comparar el paper de mida del cos a través de les tres poblacions diferents i la seva influència de cada part en el cicle de vida. Era la primera vegada que un IPM es desenvolupava per a una espècie de sargantana. He utilitzat els resultats del IPM per fer prediccions de les conseqüències demogràfiques de les variacions de mida corporal.

DISCUSSIÓ

Els ecologistes i els biòlegs evolutius tenen com a objectiu comprendre els processos que determinen la diversitat i distribució dels organismes i la forma en què interactuen amb el medi ambient. Sota el ràpid ritme dels canvis globals, la velocitat a la qual els individus responen als canvis ambientals s'ha convertit en un tema d'especial interès. La recent evidència sobre canvis fenotípics i genètics causats per pressions de selecció relacionades amb l'home suggereix que les respostes evolutives ocorren més ràpid del que abans es pensava. L'enfocament unificat de l'eco-evolució és, en aquest moment, un camp de ràpid creixement basat en múltiples enfocaments, els estudis d'eco-evolució són els més exigents quant a l'ús de dades i malgrat que s'ha desenvolupat un marc teòric robust (Fussmann et al, 2007;.), hi ha certa absència d'estudis empírics que omplen la bretxa entre les dades i les prediccions teòriques (Kasada, Yamamichi, i Yoshida, 2014;. Whitham et al, 2006). Una raó important

darrere de la falta d'estudis empírics eco-evolutius és la dificultat per a un seguiment a llarg termini dels trets dels individus i les dades d'història de vida. En aquesta tesi vaig integrar enfocaments ecològics, evolutius i genètics per investigar les estratègies d'història de vida en tres poblacions de sargantanes insulars.

La majoria dels estudis sobre els processos eco-evolutius es duen a terme en laboratoris o en sistemes controlats o semi-naturals. No obstant això, en els laboratoris la variabilitat natural és absent per definició i les pressions selectives són només les imposades pels investigadors. D'altra banda, el control dels individus en condicions naturals és difícil i part de la informació es perd inevitablement. A més, cada població és única i té una estructura d'edat i distribució de sexes particular que poden canviar amb el temps i aquesta estructura governarà la dinàmica de la població. Aquest context demogràfic és un agent important de regeneració ecològica evolutiva que s'ha de considerar, a la llum de la velocitat dels canvis evolutius.

Durant el meu estudi de tres poblacions insulars de sargantana balear (*Podarcis lilfordi*) he investigat un mètode fiable per la identificació del sexe, l'estimació d'edat i el reconeixement d'individus de les diferents poblacions (capítols I, II i III). Després he utilitzat la informació recopilada per abordar els problemes eco-evolutius sobre el creixement individual (Capítols II i III) i per investigar el paper de la mida corporal en les tàctiques d'història de vida de les sargantanes mitjançant la comparació de la relació funcional entre la seva longitud (SVL) i trets demogràfics a través de tres poblacions (capítol IV). Finalment, he utilitzat la informació genètica per inferir les històries de població i possibles pressions selectives del passat (capítol IV).

Els avenços metodològics

Durant la tesi vaig tenir la necessitat de superar alguns obstacles metodològics vinculats amb el reconeixement individual i els estudis de població per obtenir dades fiables per fer front a qüestions biològiques. Discuteixo breument els principals problemes d'aquests avenços metodològics.

El reconeixement individual: una nova tècnica no invasiva

El marcatge individual va permetre desenvolupar importants coneixements en ecologia animal i evolució (Lebreton i Nord, 1993). No obstant això, depenent del tipus, les marques poden afectar negativament el comportament i la supervivència dels individus marcats. Aquest tipus de marcatge ja no està recomanat (May 2004). L'ús d'un software a mida, l'APHIS (Suite automatitzat d'identificació fotogràfica;. Moya et al 2015), va permetre identificar amb èxit els individus a partir d'una imatge digital de les escates ventrals (vegeu el capítol I.A).

Vaig reunir un total de 5000 imatges d'una mitjana de 800 individus per població. Tot i que tenim un gran repositori d'aquesta mena, l'APHIS va ser capaç d'identificar correctament les noves imatges d'animals ja coneguts.

Estimació de la densitat de la població

La densitat de població és un paràmetre fonamental en els estudis ecològics, però, en poblacions naturals, és impossible dur a terme recomptes exhaustius. Moltes de les eines analítiques i metodològiques han estat desenvolupades per resoldre aquest problema. La comparació relativa de la densitat per diferents mètodes de mostreig va ser útil per obtenir una estimació precisa de la mida de la població i per seleccionar el mètode més adequat per estudiar les poblacions de sargantanes insulars. Encara que amb resultats comparables, el mètode per

transsectes lineals (LT) va ser el mètode que va resultar amb major variació de l'estimació a causa de la variació entre els hàbitats i les sortides inevitables per l'aplicació del model. El mètode captura-marcatge-recaptura (CMR) sense la informació espacial proporcionen estimacions amb petita variació però només en sessions múltiples. Oposadament es va demostrar que les estimacions lliurades pels models espacials explícits de captura i recaptura (SECR) donen una variància que era independent del nombre d'estimacions. Per tant, SECR és un mètode millor quan el nombre d'ocasions és limitat, mentre que CMR és més precisa si el nombre d'ocasions és gran. Des de l'altre costat, LT és adequat on l'hàbitat és més uniforme o amb espècies menys mòbils.

Avaluació del sexe i l'edat

Els adults de les sargantanes Balears (*P. lilfordi*) poden ser sexats en la captura mitjançant la inspecció visual dels porus femorals en els mascles (Dellinger et al., 1990). No obstant això, els juvenils (aprox. <50mm), i a vegades les sargantanes més grans, pot ser complicat determinar el sexe. Lecomte (1992) va suggerir que el nombre de files d'escames pot ser diferent entre mascles i femelles i es pot utilitzar per determinar el sexe d'animals juvenils.

Per tant, vaig avaluar la diferència en files d'escames ventrals per cada població emprant mascles i femelles conegudes. Les sargantanes mascles van tenir de mitjana un menor nombre de fileres d'escames ventrals (Vegeu el capítol 2). Les sargantanes amb més de 28 fileres d'escames eren sempre les femelles i els que tenen menys de 26 eren sempre mascles. És probable que la fila d'escames adicional de les femelles es degui a la necessitat d'espai durant el desenvolupament de l'ou. Amb aquesta metodologia "personalitzada" per a cada població era possible determinar el sexe al voltant del 75% dels individus.

Per altra banda, en les poblacions naturals, llevat que els animals estiguin marcats en el naixement, l'edat de l'individu és desconeguda. Per tant, relacionar la mida individual amb una etapa del cicle de vida és molt difícil. Les dades de captura-recaptura a llarg termini (vegeu el capítol I) van ser utilitzades per estimar la corba de creixement de la talla per edat (Schnute, 1981; Baker et al 1991, Somers., 2004), l'edat de maduresa sexual per ambdós sexes (capítol II) i les diferències entre les poblacions (capítol III).

Demografia evolutiva de la sargantana balear

L'efecte illa

Meiri (2007) va qüestionar si les illes són hàbitats limitats en quant a aliments i si les sargantanes petites tendeixen a ser més grans a ambients insulars. Andrews (1976) va assenyalar que el creixement de les sargantanes a les illes limitades pels aliments era més lent que la població continental o d'altres illes amb més recursos. Ell va estudiar les diferències de creixement entre les poblacions insulars i continentals de *anolis* utilitzant la taxa de creixement característic (K) (von Bertalanffy, 1969), un paràmetre que mesura el temps que triga l'individu per aconseguir la seva grandària final.

Les nostres dades donen suport a la teoria d'Andrews (1976), el creixement més baix (*K*) és en la població més petita i limitada respecte als aliments, ES (capítol III). Això indica que el creixement està limitat per la disponibilitat de recursos, però aquesta limitació no impedeixi assolir grans mides. Comparant els nostres resultats amb els d'altres poblacions de *Podarcis* tant a la part continental com a illes, es va trobar que les sargantanes de l'illa creixen més lentament que els seus parents del continent. Aquest "efecte illa" també és sostingut per la diferència dins de la mateixa espècie . Per tant, com suggereix Meiri (2008), les sargantanes no segueixen l'efecte illa com es pensava, ja que, tot i que

les poblacions insulars són 10 vegades més denses que les poblacions del continent (Buckley i Jetz, 2007), la mida no es veu afectada i les espècies de grans dimensions es fan encara més grans a les illes, només que les sargantanes insulars necessiten més temps per arribar-hi.

En espècies amb creixement continu com els rèptils, els factors de densitat-dependents i independents han demostrat que influeixen en la taxa de creixement individual durant tot el seu cicle de vida, però el seu paper relatiu és diferent segons el sexe o edat de l'individu (Andrews, 1976; Ballinger i Congdon, 1980 ; Coulson et al, 2001 ;. Galliard, François, marquès, i Massot, 2010; Massot, Clobert, Pilorge, Lecomte, i Barbault, 1992; M Mugabo, marquès, Perret, i Le Galliard, 2010; Marianne Mugabo, marquès, Perret, i Le Galliard, 2011; Marianne Mugabo, Perret, Legendre, i Galliard, 2013; Van Valen, 1965). LeGaillard et al (2010) va mostrar que una taxa de creixement era depenent de l'ambient i és el mecanisme subjacent en les diferències entre les cohorts. No obstant això, en el capítol III també es va afegir la densitat com un factor important a tenir en compte, el que suggereix que una complexa interacció entre factors extrínsecs (climàtics) i intrínsecs (densitat) governen sobre la variabilitat de la taxa de creixement del cos.

Divergències genètiques entre les illes, diferències de supervivència i fecunditat entre les illes.

A més de les diferències trobades en els paràmetres de creixement entre les poblacions insulars i continentals i els efectes de la densitat i els factors climàtics sobre la taxa de creixement en diferents edats d'una població, les divergències observades en les nostres tres poblacions d'estudi en els trets d'història de vida eren encara grans, tenint en compte que tres poblacions són de la mateixa subespècie (Salvador, 1986) i pertanyen al mateix grup genètic (Terrassa, 2009). Quan vaig aprofundir més en els seus trets d'història de vida, com ara la taxa de supervivència i fecunditat, he trobat diferències significatives entre

poblacions. En primer lloc, la població del NG està composta pels individus (mascles i femelles) 10 mm més petits que les de les altres dues illes.

La mida del cos és un conductor important de les característiques d'història de vida i la seva importància pot informar sobre la variabilitat de les pressions selectives (Peters, 1986). Per tant, la diferència de mida entre les poblacions pot influir en la supervivència i la taxa de fecunditat.

NG té la menor taxa de supervivència per als mascles i femelles en les tres poblacions (capítol III). No obstant això, NG no és ni la que compta amb les poblacions més petites i limitades pels aliments ni la més densa, que són dos factors que poden afectar la taxa de supervivència. Sorprenentment, ES es caracteritza per una composició vegetal més pobra i una densitat de població alta en comparació amb les altres dues poblacions que tenien la probabilitat de supervivència anual més alta, seguit de MO, l'illa més gran i menys densa. Els trets reproductius són imprescindibles per portar a terme estudis eco-evolutius. No obstant això, és també un dels més complicats d'obtenir per a petits vertebrats. D'altra banda la complexitat augmenta quan hi ha una mena de poligàmia com a aquestes sargantanes. Es va utilitzar un enfocament experimental per estimar la fecunditat d'aquestes (capítols III i IV).

L'anàlisi de la fecunditat va mostrar que les femelles de MO van pondre més ous que a ES i NG, però, les femelles de NG van pondre ous sent les femelles de mida inferior, una possible explicació pot ser que aquestes a NG aconsegueixen la maduresa sexual a mides més petites, a més el nombre d'ous posats en NG va ser constant i no va variar amb la mida (capítol IV). Les femelles de NG poden tenir una inversió reproductora més alta a una edat més jove. És aquesta inversió en la reproducció sent més joves la causa de l'alta mortalitat en NG o ha de ser atribuït a un component genètic? Les sargantanes mostren un notable grau de diferenciació morfològica i trets d'història de vida en aquestes tres

poblacions i les causes d'aquesta divergència poden ser diverses. Utilitzant 15 marcadors neutres vaig descobrir que la divergència genètica entre les tres poblacions no coincideix amb cap de les característiques morfològiques o les diferències ecològiques entre els illots (capítol III). Les dades dels microsatèl·lits van agrupar NG i MO en un sol grup. Encara que NG pertany al mateix grup genètic que MO, la diversitat al·lèlica de NG és molt menor. El resultat més sorprenent va ser el baix nombre d'al·lels privats en NG en comparació amb els altres illots (MO i ES). Les anàlisis de simulació que integren diferents escenaris possibles (capítol III) van suggerir una reducció important en la grandària efectiva de la població que data de fa aproximadament 620 generacions (c. 2200 anys abans del present). Això coincideix amb la data dels assentaments humans a l'illa (al voltant de 400 aC; Guerrero i Gornés, 2000). La influència dels assentaments humans passats va poder canviar les pressions selectives en NG, la introducció de depredadors per aquests pobladors pot haver seleccionat l'individu de mida més petita, forçant a la població a reproduir-se abans provocant que la inversió de la reproducció precoç afectaria la supervivència en els individus grans, especialment les femelles.

El seguiment de la dinàmica de la mida corporal

Els individus a NG eren de mitjana un 10-15% menor que als altres illots. Tot i que la causa d'aquesta reducció no s'ha demostrat directament, l'anàlisi de coalescència va suggerir que això podria ser el resultat de la presència humana en el temps històric. Tal reducció podria haver generat (o està relacionat amb) altres canvis de trets d'història de vida. Per investigar això era necessari mesurar el paper de la mida del cos dins del cicle de vida de les sargantanes a través d'un model demogràfic en el qual es van expressar els paràmetres com una funció de la grandària del cos.. Mentre que la fertilitat és constant durant la vida útil, sembla que la mortalitat va augmentar amb la mida de la femella (i edat) en NG. El patró oposat es va trobar en MO i ES, on les sargantanes més grans

semblen tenir avantatges a causa de l'efecte positiu de la mida en la fecunditat i l'aparent absència de cost de supervivència. Les anàlisis d'elasticitat confirmen que el creixement i la supervivència van ser els trets més importants de MO i ES afavorint la selecció de femelles més grans en el sistema. Per contra, en la selecció de NG es defineix per la fertilitat en les mides petites que sembla compensar la mortalitat dels individus més grans. El creixement en les tres poblacions va ser bastant estable ($\lambda \approx 1$), tenint NG el valor més alt (1.12), això vol dir que la població creixi 12%. La inversió de la reproducció precoç i la fecunditat constant augmenta el creixement de la població, tot i l'alta mortalitat soferta pels individus de grans dimensions. Per tant, la selecció afavorirà als individus més petits en el cas NG.

Tingueu en compte la importància de considerar els efectes de l'actual, així com de la presència humana en el passat com una força evolutiva addicional en la conformació de les poblacions naturals. Aquesta tesi mostra, com una possible explicació, com els efectes d'un assentament humà fa més de 2000 anys pot alterar les característiques del cicle de vida d'una població insular. No obstant això, encara que aquesta hipòtesi no ha estat avaluada directament, el que si es mostra directament aquí, és com els processos evolutius i demogràfics han afectat les respostes individuals de manera diferent causant una ràpida selecció. La selecció natural actua ràpid i pot prendre diferents direccions; per tant, cal integrar una informació lo més acurada possible sobre l'ecologia i el context demogràfic per a l'estudi d'aquests canvis evolutius tan ràpids.

CONCLUSIONS

1. El marcatge individual permet recopilar informació detallada per fer front a preguntes sobre els estudis d'ecologia de poblacions, evolutius i demogràfics. Els possibles mètodes de marcat clàssics

són reemplaçats lentament pels nous procediments no invasius per a la identificació individual. El desenvolupament d'APHIS ha permès obtenir un conjunt de dades gran i fiable d'informació basada en els individus a través de les històries de captura en una rutina semi-automàtica evitant el clàssic tall de dits.

2. Els models que tinguin en compte rang de mobilitat individual i moviments són una eina fiable per estimar les mides de població. Lliuren estimacions precises sense importar el nombre d'ocasions. Els transsectes i els mètodes de captura-recaptura clàssics sense informació espacial lliuren estimacions de la grandària de la població amb una gran variància i la precisió variable.
3. Els paràmetres de creixement van ser diferents entre els sexes i poblacions. Els mascles van aconseguir mides més grans que les femelles en totes les poblacions, però el dimorfisme sexual de mida canvia d'acord amb la població considerada. Els animals aconsegueixen arribar a la mida asimptòtica més lentament a l'illa més petita, suggerint que el paràmetre característic de creixement depenia de la disponibilitat d'aliments. Els paràmetres de creixement van tenir conseqüències sobre la maduresa sexual i la supervivència dels individus, suggerint equilibri entre el creixement, la supervivència i la reproducció.
4. Tot i que la densitat als hàbitats insulars és més gran que a la part continental, la mida del cos no es veu afectada significativament per l'entorn insular com en el cas de les poblacions de mamífers o aus. D'altra banda, el creixement característic va mostrar una notable diferència entre l'illa i les poblacions del continent a causa dels recursos limitats de moltes illes.
5. La competència despòtica en les poblacions de sargantanes insulars afecta en gran mesura a les característiques del cicle de vida dels juvenils. La taxa de creixement dels juvenils es veu

limitada per l'alta densitat de població i també per la falta de pluges. Les restriccions d'aigua afecten els individus de primer any també. Sent els individus adults els més beneficiats quan les condicions no són bones. Aquesta interacció entre covariables i mida és probablement la raó de la variabilitat temporal d'estructura de la mida de les poblacions.

6. L'estudi de l'estructura genètica dels tres illots va revelar un efecte d'aïllament per distància, sent la població més lluny la genèticament més diferent. D'altra banda, les poblacions de MO i NG pertanyen a la mateixa reserva genètica, tot i que NG té una riquesa d'al·lels menor.
7. L'ús de programes d'inferència bayesiana ha estat fonamental en el descobriment d'una important reducció en la densitat de la població de NG. El moment d'aquesta reducció es va dur a terme aparellat amb un assentament humà històric en NG durant 400 anys fa 2500 anys. Tot i que aquests resultats no són concloents, accentuen la importància de tenir en compte l'empremta humana en estudis evolutius i ecològics.
8. Les femelles més grans a NG van patir una mortalitat elevada en comparació amb les femelles grans en altres illots. En NG les femelles semblaven compensar aquesta alta mortalitat amb una reproducció precoç.
9. L'anàlisi de l'elasticitat va mostrar un contrast de la mida del cos en les tres poblacions. Les femelles grans en ES i MO tenen un avantatge selectiu, mentre que a NG la fecunditat de les femelles reproductores més petites és important per al creixement de la població.
10. Les tres poblacions insulars van mostrar un alt grau de trets d'història de vida i de divergència genètica. Aquestes diferències són probablement el resultat final dels processos de selecció particulars que ha viscut cada població.